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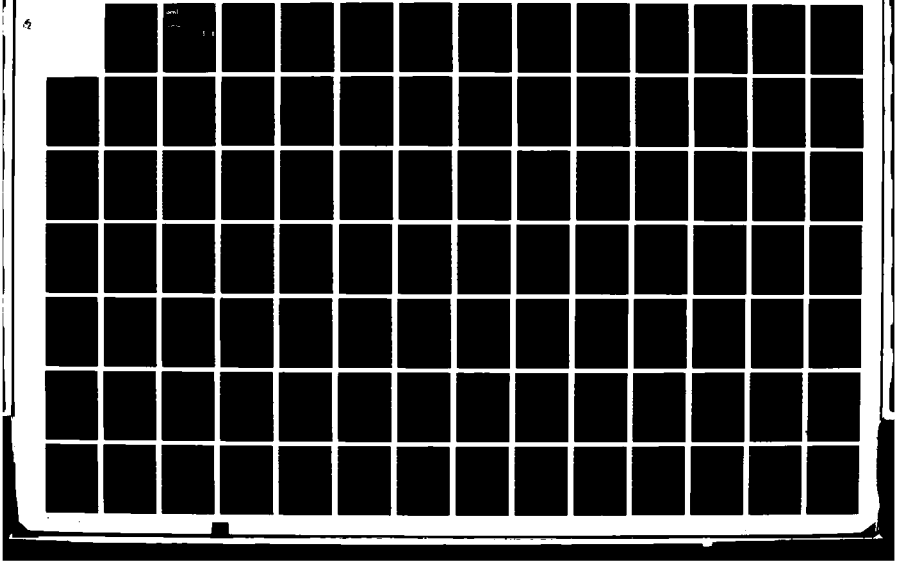

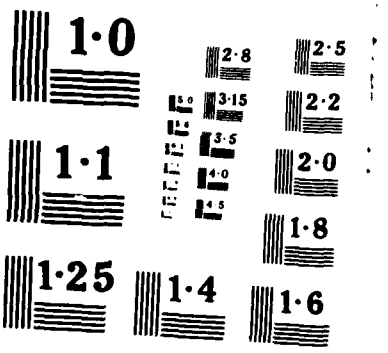
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Water Quality Criteria for 2,4-Dinitrotoluene and 2,6-Dinitrotoluene

FINAL REPORT

Elizabeth L. Etnier

August 1987

SUPPORTED BY

U.S. ARMY MEDICAL RESEARCH AND
DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701-5012
Project Order No. 84PP4845

Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831

Contracting Officer's Representative
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U.S. ARMY BIOMEDICAL
RESEARCH AND DEVELOPMENT LABORATORY
Fort Detrick, Frederick, MD 21701-5010

OPERATED BY
MARTIN MARIETTA ENERGY SYSTEMS, INC
FOR THE UNITED STATES
DEPARTMENT OF ENERGY

Printed in the United States of America. Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road, Springfield, Virginia 22161
NTIS price codes—Printed Copy: A08; Microfiche A01

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release Distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) ORNL-6312	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Oak Ridge National Laboratory 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Chemical Effects Information Task Group Biology Division Oak Ridge National Lab., Oak Ridge, TN 37831-6050	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research and Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER APO 84PP4845
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62770A; PROJECT NO. 3M162770-A870; TASK NO. AI; WORK UNIT ACCESSION NO. 021

11. TITLE (Include Security Classification)

Water Quality Criteria for 2,4-Dinitrotoluene and 2,6-Dinitrotoluene (DNT)

12. PERSONAL AUTHOR(S)

Elizabeth L. Etnier

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM _____ TO _____	1987 August	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	13	
06	09	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
aquatic toxicity, environmental effects, environmental fate, 2,4-DNT, 2,6-DNT, health effects, mammalian toxicity, metabolism, munition products, water quality criteria,

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Data available for calculating a Final Acute Value (FAV) for 2,4-DNT do not meet all the requirements specified by the USEPA guidelines. However, since the data generated by these toxicity tests are uniform in their assessment of the degree of toxicity of 2,4-DNT, a freshwater FAV of 11.02 mg/L 2,4-DNT was estimated. Although insufficient data are available to calculate a criterion as described in the USEPA guidelines, using the FAV and an acute/chronic ratio of 54.3, an interim Criterion Maximum Concentration (CMC) of 5.5 mg/L 2,4-DNT and a Criterion Continuous Concentration (CCC) of 0.20 mg/L 2,4-DNT are suggested. At present, no CMC or CCC can be calculated for 2,6-DNT; however, based on only a few studies, it appears that the 2,6- isomer is approximately twice as toxic to aquatic organisms as the 2,4- isomer.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian	22b. TELEPHONE (Include Area Code) (301) 663-7325 22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.*

SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

19. ABSTRACT (continued)

Based on the evidence of an increased incidence of hepatic carcinomas and hepatic neoplastic nodules in male rats, the recommended criteria to achieve a human health risk of 10^{-5}, 10^{-6}, or 10^{-7} for 2,4-dinitrotoluene are 1.7, 0.17, and 0.017 μg/L, respectively. It should be noted that the 2,4-DNT used in the bioassay from which the criteria were calculated was 98 percent pure, with the remaining 2 percent comprised of predominantly 2,6-DNT. The possible influence of 2,6-DNT on the results of this study should not be overlooked. Results from a tumor bioassay suggest that pure 2,4-DNT is not carcinogenic, but limitations of the study preclude a definitive statement regarding the carcinogenicity of 2,4-DNT, There are no studies available documenting the systemic toxicity of pure 2,4-DNT, and thus no acceptable daily intake can be calculated.

The water quality criterion for 2,6-DNT is derived from the data showing an increased incidence of hepatic carcinomas in male Fischer 344 rats. It should be noted that exogenous factors in the diet can affect the carcinogenicity of the DNT isomers, enhancing the metabolism and hepatic covalent binding of 2,6-DNT in particular. However, 2,6-DNT is unquestionably a potent hepatocarcinogen, and criteria based on this study will give a conservative estimate of the acceptable cancer risk.

The recommended criteria to achieve a risk of 10^{-5}, 10^{-6}, or 10^{-7} for 2,6-dinitrotoluene are 68.3, 6.8, and 0.68 ng/L, respectively.

ORNL-6312

Water Quality Criteria for 2,4-Dinitrotoluene and 2,6-Dinitrotoluene

FINAL REPORT

Elizabeth L. Etnier

Chemical Effects Information Task Group
Information Research and Analysis
Biology Division

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Date Published — August 1987

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U.S. DEPARTMENT OF ENERGY
Under Contract No. DE-AC05-84OR21400

EXECUTIVE SUMMARY

2,4-Dinitrotoluene (DNT) (CAS No. 121-14-2) and 2,6-DNT (CAS No. 606-20-2) are used as ingredients of military and commercial explosives, as well as intermediates in the commercial production of polyurethanes. A somewhat purified form of 2,4-DNT is used in smokeless powders. All six possible isomers of DNT occur as by-products during the incomplete trinitration of toluene and are released in aqueous effluents resulting from trinitrotoluene (TNT) manufacture and cleanup.

Biodegradation of 2,4-DNT and 2,6-DNT occurs under anaerobic conditions in the presence of an exogenous carbon source; a slight biodegradation of 2,6-DNT was found to occur under aerobic conditions. About 50 percent of 2,4- or 2,6-DNT found in TNT condensate water was lost after exposure to sunlight for 5 days and 1 day, respectively. The major metabolite of photolyzed 2,4-DNT is 2,4-dinitrobenzoic acid, with the ultimate photolysis products carbon dioxide, water, and nitric acid.

Forty-eight hour EC_{50} values for 2,4-DNT range from 26.2 to 47.5 mg/L for daphnids. Freshwater fish appear to be more sensitive to 2,4-DNT than invertebrates, with LC_{50} values of about 13 mg/L for bluegills and rainbow trout, and 24.8 and 32.8 mg/L for channel catfish and fathead minnows, respectively. Based on limited information, it appears that 2,6-DNT may be about twice as toxic to daphnids and fathead minnows as 2,4-DNT. Photolysis and oxidation were found to greatly reduce 2,4-DNT toxicity. Temperature variations were also found to affect toxicity, with 2,4-DNT being two and three times as toxic at 20 and 25°C, respectively, as at 17°C.

Chronic exposure of daphnids to 2,4-DNT results in reproductive effects at a concentration of 0.40 mg/L. Significant histopathological changes are seen in bluegill sunfish during chronic exposure to 0.5 mg/L 2,4-DNT, and egg production is significantly reduced in fathead minnows during a 6-month exposure to 0.62 mg/L 2,4-DNT. Growth effects are seen in rainbow trout at concentrations slightly above these. No information was found documenting the chronic toxicity of 2,6-DNT to freshwater organisms, or the acute or chronic toxicity of 2,4- or 2,6-DNT to saltwater organisms.

Data available for calculating a Final Acute Value (FAV) for 2,4-DNT do not meet all the requirements specified by USEPA guidelines; i.e., only seven of the appropriate families of aquatic test animals have been used in acute LC_{50} tests rather than the eight families required by the guidelines. However, since the data generated by these toxicity tests are uniform in their assessment of the degree of toxicity of 2,4-DNT, a freshwater FAV of 11.02 mg/L 2,4-DNT was estimated. Although insufficient data are available to calculate a criterion as described in the USEPA guidelines, using the FAV and an acute/chronic ratio of 54.3, an interim Criterion Maximum Concentration (CMC) of 5.5 mg/L 2,4-DNT and an interim Criterion Continuous Concentration (CCC) of 0.20 mg/L 2,4-DNT are suggested. At present, no CMC or CCC can be calculated for 2,6-DNT; however, based on only a few studies, it appears

that the 2,6- isomer is approximately twice as toxic as the 2,4- isomer to aquatic organisms.

2,4-DNT absorbed from the intestine of laboratory animals is principally metabolized by the liver to dinitrobenzyl alcohol (DNBAlc), which is conjugated to form dinitrobenzyl alcohol glucuronide (DNBAlcG). In female rats, the DNBAlcG is primarily excreted in the urine, while in male rats biliary excretion of DNBAlcG predominates. In males or females, the DNBAlcG that is excreted in the bile returns to the intestine, where it is deconjugated by intestinal microflora to DNBAlc. This DNBAlc may then be further metabolized by the microflora to an active metabolite, which is absorbed once again and is covalently bound to hepatic macromolecules. The concentration of metabolites is higher in livers of male rats than in livers of female rats. Hepatic binding of 2,6-DNT in protein, RNA, and DNA is 2 to 5 times greater than that of 2,4-DNT. The principal route of excretion of 2,4- and 2,6-DNT and their metabolites is via the urine, with excretion virtually complete after 24 hr.

In humans working in explosives factories, there is no substantial evidence of accumulation of 2,4- or 2,6-DNT metabolites from day to day, and there is no apparent difference in the time course of elimination of metabolites. The presence of 2-amino-4-nitrobenzoic acid and 2-acetylamino-4-nitrobenzoic acid as major metabolites in human urine indicates that nitro group reduction is occurring in humans. Although the metabolites identified in human urine are qualitatively similar to those identified in rat urine, there are quantitative differences in the metabolites measured.

Rats appear to be more susceptible to 2,4-DNT toxicity than mice, with oral LD_{50} values for rats and mice ranging from 268 to 650 mg/kg and 1250 to 1954 mg/kg, respectively. Male rats are more sensitive to 2,4-DNT than female rats. Male rats are also more sensitive than females to 2,6-DNT than to 2,4-DNT toxicity. Both sexes of mice are more sensitive to the 2,6- isomer than the 2,4- isomer, with oral LD_{50} values for both sexes ranging from 621 to 1000 mg/kg 2,6-DNT. Toxic signs resulting from acute exposure to either isomer are central nervous system depression resulting in ataxia, respiratory depression, and death within 24 hr. 2,4-DNT was found to induce methemoglobinemia and sulfhemoglobinemia in rats, with reticulosis and the presence of Heinz bodies.

Subchronic feeding with 2,4-DNT was lethal to 50 percent of beagle dogs fed 25 mg/kg/day, with neuromuscular effects, methemoglobinemia and Heinz bodies, anemia, reticulocytosis, extramedullary hematopoiesis, and reduced spermatogenesis. A dose of 5 mg/kg/day 2,4-DNT is reported as a no observed effects level (NOEL). Doses of 20 mg/kg/day 2,6-DNT resulted in 25 percent lethality in dogs and clinical signs similar to those of 2,4-DNT, including lesions in the liver and spleen. A dose of 4 mg/kg/day 2,6-DNT caused mild splenic hematopoiesis. No NOEL was reported for 2,6-DNT in dogs.

Rats exhibited the same clinical signs as a result of 13 weeks exposure to 2,4-DNT as dogs, with a lowest observed adverse effects level (LOAEL) of 34 to 38 mg/kg/day 2,4-DNT. Severe testicular atrophy and aspermatogenesis were reported at 93 mg/kg/day. An NOEL of 7.2 to 7.4 mg/kg/day 2,6-DNT is reported for rats, with 35 mg/kg/day and higher resulting in clinical signs similar to those seen with the 2,4- isomer, as well as testicular, bile duct, and splenic lesions.

Mice are more tolerant of subchronic (90 day) 2,4-DNT toxicity than dogs or rats, with an NOEL of 137 to 147 mg/kg/day, and with only mild anemia and weight loss seen at 413 to 468 mg/kg/day. However, as with dogs and rats, they seem to be more susceptible to 2,6-DNT toxicity, with an NOEL of 11.0 mg/kg/day 2,6-DNT for both sexes. Testicular atrophy, bile duct hyperplasia, and liver and splenic degenerative changes were seen at 50 to 55 mg/kg/day 2,6-DNT. Doses of 50 or 290 mg/kg/day 2,6-DNT resulted in 100 percent death of males and 12 and 75 percent death, respectively, of females.

Subchronic and chronic exposures of workers in munitions plants have been reported from the early and mid-1900s. Symptoms recorded following exposure were cyanosis, dizziness, and a tendency to sleep, with headache, dyspnea, and brown urine. Alcoholic subjects were quite sensitive to toxicity. Other symptoms included vomiting, numbness and tingling, loss of weight, and diarrhea. No fatalities were recorded.

A study of cohorts of workers exposed to DNT in two munitions plants operating in the 1940s and 1950s indicated an increase in ischemic heart disease over that seen in white males in the United States and in persons living in the vicinity of the two plants. The data suggest a correlation between mortality and length and intensity of exposure to DNT.

The genotoxicity of 2,4- and 2,6-DNT has been studied by various authors using a multitude of short-term toxicity assays. A weak mutagenicity response has been reported for the 2,4- isomer using the Ames Salmonella assay, with reduction or elimination of activity in the presence of a metabolic activation system. The 2,6- isomer gave a positive response in the Ames assay with or without metabolic activation. Neither the 2,4- nor the 2,6- isomer gave a positive result using a Salmonella strain lacking nitroreductase activity, indicating that the mutagenic response of the DNTs depends on bacterial nitroreductase activity.

2,4-DNT was found to cause chromosome aberrations and aneuploidy/cell-cycle disruptions in the RTG2 and BF2 fish cell lines, and it caused an increase in DNA single-strand break frequency using a rat hepatocyte assay. Pure 2,4-DNT was found to produce a dose-related increase in mutation frequency and a decrease in survival in the P388 mouse lymphoma assay, whereas technical grade DNT (tDNT) and 2,6-DNT produced no mutagenic effect. The V79 cell metabolic cooperation assay and the CHO/HGPRT somatic cell mutagenesis assay both proved negative for 2,4-, 2,6-, and tDNT.

Both 2,4- and 2,6-DNT were found to elicit a negative response in the in vitro unscheduled DNA synthesis (UDS) assay. However, in vivo-in vitro UDS assays demonstrated that both isomers produce genotoxic responses, with the 2,6- isomer an order of magnitude more toxic than the 2,4- isomer.

No positive correlation was established between 2,4-DNT and reproductive effects observed among workers in a DNT factory, or reported in rat or mouse studies using 2,4- or tDNT. A high maternal and embryo/fetal toxicity was observed, however, in laboratory animal studies. Dominant lethal assays proved negative for 2,4-DNT.

2,4-DNT was not found to be carcinogenic in male or female Fischer 344 rats following daily intake of 0.02 or 0.008 percent 2,4-DNT in the diet for 18 months. In male rats there was a significant increase in skin and subcutaneous tissue fibromas, and in female rats there was an increase of fibroadenoma of the mammary gland. Similarly, 2,4-DNT was not found to be carcinogenic in beagle dogs following administration of up to 10 mg/kg/day for 2 yr.

However, the progressive development of hepatocellular carcinoma was reported in Charles River CD rats, with 66 percent of the females and 27 percent of the males exhibiting hepatic neoplastic nodules or hepatocellular carcinomas following oral doses of 45 and 35 mg/kg/day, respectively, 2,4-DNT for more than a year. In addition to hepatic tumors, there was also a greatly increased incidence of benign subcutaneous tumors.

In 2-yr feeding studies with Charles river CD-1 mice, the high dose of 2,4-DNT (900 mg/kg/day) resulted in nearly 100 percent lethality. Although no hepatic tumors were observed, a greater incidence of kidney tumors was seen in middle dose (95 mg/kg/day) male mice after 24 months compared to controls.

In a study designed to test the carcinogenic effects of 2,4-, 2,6-, and tDNT, 47 percent of male Fischer 344 rats fed 35 mg/kg/day tDNT (18.8 percent 2,6-DNT) for 1 yr developed hepatocarcinomas, compared with 85 and 100 percent for rats fed 7 and 14 mg/kg/day pure 2,6-DNT, respectively, and none for rats fed 27 mg/kg/day pure 2,4-DNT. This indicates that most of the carcinogenicity of tDNT can be attributed to the 2,6- isomer. Although these results suggest that pure 2,4-DNT is not carcinogenic, limitations of the study preclude a definitive statement regarding the carcinogenicity of 2,4-DNT, and the USEPA weight-of-evidence classification is best considered to be Group D.

Increased pectin in the diet of Fischer 344 rats has been shown to cause an increased incidence of hepatic carcinomas following ingestion of 3.5 mg/kg/day 2,6-DNT for 1 yr. In contrast, rats fed diets low in pectin did not develop any tumors or neoplastic nodules after feeding with 3.5 mg/kg/day 2,6-DNT for 1 yr. The possibility of a causal relationship between carcinogenicity and other factors in the diet has been suggested.

The recommended criteria to achieve a human cancer risk of 10^{-5}, 10^{-6}, or 10^{-7} for 2,4-DNT are 1.7, 0.17, and 0.017 µg/L, respectively. It should be noted that the 2,4-DNT used in the bioassay from which the criteria were calculated was 98 percent pure, with the remaining 2 percent comprised of predominantly 2,6-DNT. By analogy to other studies described above, the possible influence of 2,6-DNT on the results of this study should not be overlooked. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of contaminated water, the levels are 30.0, 3.0, and 0.30 µg/L, respectively. Based on the interim Criterion Maximum Concentration estimated for aquatic life (5.5 mg/L 2,4-DNT), and the interim Criterion Continuous Concentration (0.20 mg/L 2,4-DNT), maintenance of concentrations at the levels recommended for the human health criteria would more than adequately protect aquatic life.

The water quality criterion for 2,6-DNT is derived from the data showing an increased incidence of hepatic carcinomas in male Fischer 344 rats. It should be noted that exogenous factors in the diet can affect the carcinogenicity of the DNT isomers, enhancing the metabolism and hepatic covalent binding of 2,6-DNT in particular. However, 2,6-DNT is unquestionably a potent hepatocarcinogen, and criteria based on this study will give a conservative estimate of the acceptable cancer risk.

The recommended criteria to achieve a risk of 10^{-5}, 10^{-6}, or 10^{-7} for 2,6-dinitrotoluene are 68.3, 6.8, and 0.68 ng/L, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 1.2, 0.12, and 0.012 µg/L, respectively.

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1. INTRODUCTION

Dinitrotoluene (DNT) occurs in six isomeric forms. 2,4-DNT (CAS No. 121-14-2) and 2,6-DNT (CAS No. 606-20-2) are the isomers of concern in this report. The principal application of DNT is as an intermediate in the manufacture of toluene diisocyanate (Dunlap 1978), and thence polyurethanes (Hunt et al. 1980). It is used to a lesser extent as an intermediate in dye processes and as an ingredient of military and commercial explosives (Hunt et al. 1980). This latter application is due to the gelatinizing and waterproofing action of DNT, as well as its explosive potential (Fishbein 1979). A relatively pure form of 2,4-DNT (about 96 percent 2,4-DNT) is used for smokeless powders (Urbanski 1984).

1.1 PHYSICAL AND CHEMICAL PROPERTIES

All isomers of DNT are pale, yellow, crystalline products (Urbanski 1983). 2,4-DNT occurs as crystalline needles, and is very slightly soluble in water, 0.03 g/100 g at 22°C (Dean 1979). It is soluble in alcohol, ether, acetone, and benzene (Weast 1984), with solubilities of 1.2 and 9 g/100 g at 15°C for alcohol and ether, respectively (Dean 1979). The USEPA (1980a) lists the solubility of 2,4-DNT in carbon disulfide at 17°C as 21.9 g/L and the solubility in diethyl ether at 22°C as 94 g/L. 2,6-DNT is found in the form of rhombic needles (Weast 1984), and is listed as soluble in alcohol (Dean 1979).

A summary of the physical and chemical properties of 2,4-DNT and 2,6-DNT is given in Table 1.

Dinitrotoluenes form molecular compounds with condensed ring aromatic hydrocarbons and with amines. The methyl group in dinitrotoluenes (Table 1) is very reactive, due to the presence of the two nitro groups (Urbanski 1983). The dinitrotoluenes decompose readily at elevated temperatures, and they may decompose at lower temperatures in the presence of impurities. They burn safely if unconfined, but may undergo explosion if confined (Manufacturing Chemists Association 1966).

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

The dinitration of toluene with nitric acid in the presence of concentrated sulfuric acid results in the formation of several isomeric products. Technical grade DNT (tDNT) manufactured from a mixture of toluene and sulfuric/nitric acid is typically composed of the following isomers: 78 wt% 2,4-DNT, 19 wt% 2,6-DNT, 2.5 wt% 3,4-DNT, 1 wt% 2,3-DNT, and 0.5 wt% 2,5-DNT (Dunlap 1978). In industrial practice, mononitrotoluene is nitrated with a mixture of 28 to 34 percent nitric acid, 60 to 64 percent sulfuric acid, and 5 to 8 percent water (Urbanski 1983). The nitration of o-nitrotoluene produces 2,4-DNT or 2,6-DNT, while the nitration of p-nitrotoluene produces 2,4-DNT alone. It has been generally accepted that the 2,3-, 3,4-, and 3,6-DNT (3,6- is

TABLE 1. PHYSICAL AND CHEMICAL PROPERTIES OF DINITROTOLUENE (DNT)

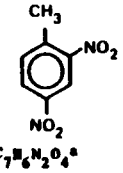
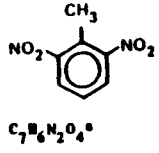
	2,4-DNT	2,6-DNT
A. Chemical identity		
1. CAS registry number	121-14-2	606-20-2
2. Chemical name	2,4-dinitrotoluene	2,6-dinitrotoluene
3. Synonyms	1-methyl-2,4-dinitrobenzene, 2,4-dinitrotoluol, DNT, and NCI-C01865[a]	2-methyl-1,3-dinitrobenzene[a]
4. Structural formula	 $C_7H_6N_2O_4$[a]	 $C_7H_6N_2O_4$[a]
5. Molecular formula	$CH_3C_6H_3(NO_2)_2$[a]	$CH_3C_6H_3(NO_2)_2$[a]
B. Physical and chemical properties		
1. Molecular weight	182.15[a]	182.15[a]
2. Physical state	Yellow needles or monoclinic plates[b]	Rhombic needles[b]
3. Melting point (°C)	70[c]	64-66[c]
4. Boiling point (°C)	300[c]	
5. Partition coefficient (log P) (octanol/water)	2.01[d] 1.98[e] 2.28[f]	
6. Density (g/cm^3)	$1.321\frac{71}{4}$[c]; $1.521\frac{15}{4}$[h]	$1.283\frac{111}{4}$[g]
7. Refractive index ($n\frac{20}{d}$)	1.442[b]	1.479[b]
8. Flash point (closed cup, °F)	404[i]	
9. Vapor density (air = 1)	6.27[h]	

TABLE 1 (CONTINUED)

	2,4-DNT	2,6-DNT
10. Vapor pressure (torr)	0.005[j]	0.018[j]
11. Heat of combustion (kcal/mole)	853.7[k]	855.2[k]
12. Heat of vaporization (cal/g)	93[i]	
13. Heat of fusion (cal/g)	26.4[d]	
14. Heat of formation (kcal/mole)	7.7[l], 31.3[m]	29.6[m]
15. Melting enthalpy (kcal/kg)	26.1[n]	22.5[n]
16. Enthalpy of formation (kcal/kg)	-89.5[n]	-57.6[n]
17. Heat of detonation (kcal/kg)	-1056[n]	-1538[n]
18. Air pollution factors	1 mg/m^3 = 0.13 ppm[c]	1 mg/m^3 = 0.13 ppm[c]
19. Odor threshold		0.1 mg/L[c]
20. Stability	Stable below 250°C[i]	
21. Henry's law constant, H_c (torr M^{-1})	3.4[j]	18[j]

a. Tatken and Lewis 1983.
b. Weast 1984.
c. Verschueren 1983.
d. USEPA 1980a.
e. Dilley et al. 1979.
f. Liu et al. 1983.
g. Dean 1979.
h. Dunlap 1978.
i. Weiss 1980a.
j. Spanggord et al. 1980.
k. Urbanski 1983.
l. Bichel 1905, as reported in Urbanski 1983.
m. Garner and Abernathy 1921, as reported in Urbanski 1983.
n. Meyer 1979, as reported in Urbanski 1984.

identical to 2.5-) isomers are produced from m-nitrotoluene; however, small amounts of these latter isomers may be formed from the nitration of the o- and p-nitrotoluenes (Urbanski 1984). Rosenblatt and Dennis (1976) were able to completely remove small quantities of 2,3-, 3,4-, and 2,5- isomers from an isomeric mixture using a small amount of an alkali metal disulfide. These isomers were converted to water-soluble reaction products and separated out, leaving 80.48 percent 2,4-DNT and 19.52 percent 2,6-DNT.

All six possible isomers of DNT occur as by-products during the manufacture of trinitrotoluene (TNT). These isomers are formed as a result of the incomplete trinitration of toluene, comprising about one percent of the finished TNT. The conventional sellite purification of TNT does not appear to remove these isomers. 2,4-DNT occurs as 0.50 percent and 2,6-DNT as 0.25 percent of the crude and finished TNT (Ryon et al. 1984). Isomers of DNT are contained in aqueous effluents resulting from TNT contact with plant cleanup and scrubber water during manufacture and as a condensate from evaporative concentration and incineration of wastewater formed during sellite purification (Ryon et al. 1984). An analysis of samples of condensate wastewater from the Volunteer Army Ammunition Plant (AAP) in Chattanooga, Tennessee, was made by Spanggord et al. (1978), who found that the DNT isomers were the dominant components in the wastewater. The average concentration of 2,4-DNT in condensate samples was 14.7 mg/L (43.4 percent) and that of 2,6-DNT was 7.3 mg/L (21.5 percent).

Several AAPs are equipped to manufacture TNT, including the Volunteer plant in Chattanooga, Tennessee; the Radford plant in Radford, Virginia; the Joliet plant in Joliet, Illinois; and the Newport plant in Newport, Indiana (Liu et al. 1983).

Various techniques are available for the analysis of DNT isomers in water, sediment, and soil samples (Shafer 1982; Krull et al. 1983; Fine et al. 1984; Weinberg and Hsu 1983; Eichelberger et al. 1983, Hartley et al. 1981). These include gas chromatography (GC) and high-pressure liquid chromatography (HPLC), in conjunction with various selective and/or general detection methods (Krull et al. 1983). Detection methods such as electron-capture detection (ECD), thermionic ionization, and thermal energy analysis have been utilized with GC analysis; and electrochemical detection, fluorescent detection, and ultraviolet (UV) absorption have been used with HPLC techniques (Maskarinec et al. 1984).

Krull et al. (1983) have detected parts per trillion (ppt) levels of 2,4-DNT and 2,6-DNT in mixtures of organic nitro compounds using a combination of photoionization detection (PID) and ECD with GC analysis. GC/ECD was used by Hartley et al. (1981) to separate and identify DNT isomers in spiked water samples, with detection limits in the picogram (pg) range.

Fine et al. (1984) report that a thermal energy analyzer (TEA) interfaced to a gas chromatograph is capable of detecting 5 pg of 2,4-DNT in standard solutions. The use of this technique, as well as the combination of a TEA with HPLC, is suggested for detection of 2,4-DNT in

explosives, explosion debris, hand swabs, and human plasma. Phillips et al. (1983) reported detection limits of 0.05 mg/L [(50 parts per billion (ppb)] using GC/TEA to determine the presence of nitroaromatics in biologically activated sludge. Minimum detection limits of 0.05 ppb for the 2,4- and 2,6- isomers using GC/ECD were reported by Shafer (1982).

Isomers of DNT were identified in seawater with the use of gas-liquid chromatography using ECD and a combined GC-mass spectrometry (MS) computer-assisted technique (Hashimoto et al. 1978). The limits of detection were 0.186 μg/L (186 ppt) for 2,4-DNT and 0.072 μg/L (72 ppt) for 2,6-DNT. Hashimoto et al. (1980) were later able to realize detection limits of 0.13 and 0.059 μg/L for the 2,4- and 2,6- isomers, respectively, using GC with glass capillary columns and ECD.

Bongiovanni et al. (1984) report a method for the preparation and analysis of explosive-contaminated soils for the detection of trace amounts of DNT. Preparation requires stabilization of soils at 20 to 30 percent moisture, homogenization, and acetonitrile extraction; analysis includes reverse-phase liquid chromatographic separation and UV spectrometric detection. Detection limits of 0.58 parts per million (ppm) for 2,4-DNT and 0.87 ppm for 2,6-DNT are possible with this procedure. Average recovery of 2,4-DNT and 2,6-DNT from spiked soil controls was 100.4 percent and 99.6 percent, respectively, for concentrations of 0.5 and 200 ppm.

A method to determine the presence of DNT vapor concentrations in workplace air has been proposed by Hunt et al. (1980). Concentrations as low as 0.1 mg/m^3 in a 50-L air sample can be reliably detected by trapping DNT vapors on silica gel solid-sorbent sampling tubes, desorption in chloroform, and GC determination of the various isomers of DNT.

2. ENVIRONMENTAL EFFECTS AND FATE

2.1 ABIOTIC ENVIRONMENTAL EFFECTS

Sullivan et al. (1977) conducted an aquatic field survey at Volunteer AAP, a TNT manufacturing plant discharging waste into Waconda Bay, a part of the Lake Chickamauga Reservoir emptying into the Tennessee River. Elevated levels of nitrobodies, nitrogen compounds, and dissolved solids were discharged in the AAP waste. Water quality data indicated that the AAP discharge in the upper portion of Waconda Bay increased the concentrations of TNT, 2,4-DNT, 2,6-DNT, total dissolved solids, carbonates, chlorides, ammonia, organic nitrogen, nitrates, and nitrites. These effects were noticeable 0.6 mile downbay from the discharge area, and persisted some 9 weeks after the sampling period (during which the plant was shut down). The enrichment effects were not detected in two nearby reference bays. The relationship between the presence of the munitions-based compounds and the elevated water quality parameters was impossible to determine.

2.2 ENVIRONMENTAL FATE

2.2.1 Migration

Pereira et al. (1979) reported the presence of 2,4-DNT in samples of groundwater taken beneath and downgradient from munitions disposal sites at the Hawthorne Naval Ammunition Depot, Nevada. GC-MS was utilized for analysis; concentrations were not reported.

Measurements of 2,4-DNT concentrations in seawater at four sampling stations were reported by Hashimoto et al. (1978). It was estimated that such tidal actions as mixing, diffusion, and transportation would dilute the DNT by one-half within a distance of about 1.5 km. No other information regarding migration of DNT isomers was found in the literature.

2.2.2 Biological Degradation

Liu et al. (1984) used a mixed bacterial culture derived from municipal activated sludge to study the biotransformation of 2,4-DNT under aerobic and anaerobic conditions. They report no breakdown of 2,4-DNT in aerobic fermentors after 14 days of incubation, but found extensive biotransformation over the same time period in anaerobic fermentors in the presence of an exogenous carbon source. Breakdown products from 5 mg/L 2,4-DNT in methanol were identified with GC-MS as 2-nitroso-4-nitrotoluene (2N4NT), 2-amino-4-nitrotoluene (2A4NT), and 4-amino-2-nitrotoluene (4A2NT). The nitrosonitrotoluene was only detectable during the first 2 to 3 days of the incubation, while the aminonitrotoluenes were detectable for almost the entire duration of the experiment. The ultimate fate of these products was not traced. Increasing the

concentration of 2,4-DNT to 25 mg/L resulted in the appearance of a fourth intermediate, 4-nitroso-2-nitrotoluene (4N2NT), in the anaerobic fermentor broth.

In contrast, Hallas and Alexander (198^) reported a slight reduction of 2,6-DNT under aerobic incubation after 28 days in primary effluent from raw municipal sewage. Under anaerobic conditions, these authors reported a 35 percent decrease in absorbance of 2,6-DNT, measured with a double-beam spectrophotometer after 14 days, and a 55 percent decrease in absorbance after 28 days. The disappearance of the compounds was attributed to microbial activity. In both the aerobic and the anaerobic studies, products different from the 2,6-DNT were identified by UV spectra. Aromatic amines and aminonitrotoluenes were detected in both instances.

The biodegradation of 2,4- and 2,6-DNT using settled domestic wastewater as the inoculum was studied by Tabak et al. (1981). They used concentrations of 5 and 10 mg/L in static culture flasks and reported the percent degradation at weekly intervals for 4 weeks (Table 2). Significant initial degradation was reported for both isomers (50 to 77 percent for 2,4-DNT and 57 to 82 percent for 2,6-DNT), with greater degradation occurring at the lower concentrations. At both concentrations studied, there was a reduction of the biodegradation rate in each successive week, and there was an accumulation of the test compound in the culture medium. The authors suggest that the reduction in rate may have been due to the possible loss of synergistic activity on the substrate as a result of the subculture method they used, or that it may have been due to a retardation of the adaptive enzyme process caused by accumulation of toxic by-products of metabolism in the inoculum.

TABLE 2. BIODEGRADATION OF 2,4- AND 2,6-DINITROTOLUENE[a,b,c]

Test Compound	Concentration (mg/L)	Percent Biodegradation[d] 1 Week	2 Weeks	3 Weeks	4 Weeks
2,4-Dinitrotoluene	5	77	61	50	27
	10	50	49	44	23
2,6-Dinitrotoluene	5	82	55	47	29
	10	57	49	35	13

a. Adapted from Tabak et al. 1981.
b. Static incubation in flasks at 25°C in the dark.
c. Settled domestic wastewater used as inoculum.
d. Average of three test flasks.

Similar results for 2,4- and 2,6-DNT were reported by Patterson and Kodukala (1981) for static flask acclimation/degradation studies. Bacterial inocula derived from settled domestic sewage were dosed with 5 mg/L and incubated for 7 days. Each 7 days thereafter, a subculture was

redosed for three successive subcultures. Seventy-seven percent of the 2,4-DNT in the original culture was biodegraded, and 27 percent was biodegraded in the third subculture, compared with 82 percent and 29 percent, respectively, for 2,6-DNT. This inhibition of biodegradation suggests toxic effects from the DNT isomers. In full-scale biological treatment systems, Patterson and Kodukala (1981) report 75 percent removal of 2000 μg/L 2,4-DNT and 76 percent removal of 1900 μg/L 2,6-DNT, using activated sludge with powdered activated carbon treatment (PACT) in both cases. Aerated lagoon treatment (ALT) of 3 μg/L of the 2,4-isomer resulted in no removal, although ALT of 12 μg/L 2,6-DNT resulted in >83 percent removal. Activated sludge alone, without PACT, had no effect on the biodegradation of 390 μg/L 2,4-DNT, suggesting that adsorption by PACT was the primary mechanism of removal.

In an earlier study, McCormick et al. (1976) studied the hydrogen reduction of 2,4-DNT in the presence of enzyme preparations from the anaerobic bacterium Veillonella alkalescens. They concluded that the para-nitro group is reduced first and most readily. They identified 4-amino-2-nitrotoluene (4A2NT) after 10 min reaction time, but no 2-amino-4-nitrotoluene (2A4NT), suggesting that most of the 4-nitro group was reduced before reduction of the 2-nitro group began. 2,4-Diaminotoluene was also identified in the reaction mixture up to an hour after initiation of the study.

Microbial transformation of 2,4-DNT was also studied by McCormick et al. (1978) using Mucrosporium sp. in culture for up to 168 hr. Thin-layer chromatography and GC/MS techniques were used to identify 2A4NT, 4A2NT, 2,2'-dinitro-4,4'-azoxytoluene (4,4'Az), 4,4'-dinitro-2,2,'-azoxytoluene (2,2'Az), and 4-acetamido-2-nitrotoluene (4Ac2NT). A third azoxy compound was isolated but not identified. Figure 1 shows the proposed pathway for the biotransformation of 2,4-DNT as reported in McCormick et al. (1978). The nitroso and hydroxylamino intermediates were not detected experimentally, but they are included as hypothetical intermediates in the pathways since the reduction of 2,4-DNT to 2A4NT and 4A2NT proceeds via these compounds. No 2,4DAT was identified during the study, but it is included in the pathway because complete reduction of both nitro groups to amino groups was reported during the biotransformation of 2,4-DNT by anaerobic bacterial systems (McCormick et al. 1976).

Parrish (1977) cultured 190 fungal species representing 98 genera in media containing glucose and 100 mg/L 2,4-DNT. He found that only five of the species were able to transform 2,4-DNT, with transformation complete within 24 hr. Transformation products and the species carrying out the transformation were not identified.

Degradation rates of 50 mg/L of 2,4- or 2,6-DNT were studied using industrial wastewater treatment sludge containing four bacteria, Acinetobacter, Alcaligenes, Flavobacterium, and Pseudomonas, and one yeast, Rhodotorula (Davis et al. 1981). Rapid degradation of 2,4-DNT occurred, with 10 and 12 mg/L measured at day 2 and day 7, respectively. Both acid and base-neutral extraction samples taken after 2 days showed the evidence of metabolites, one of which was confirmed as

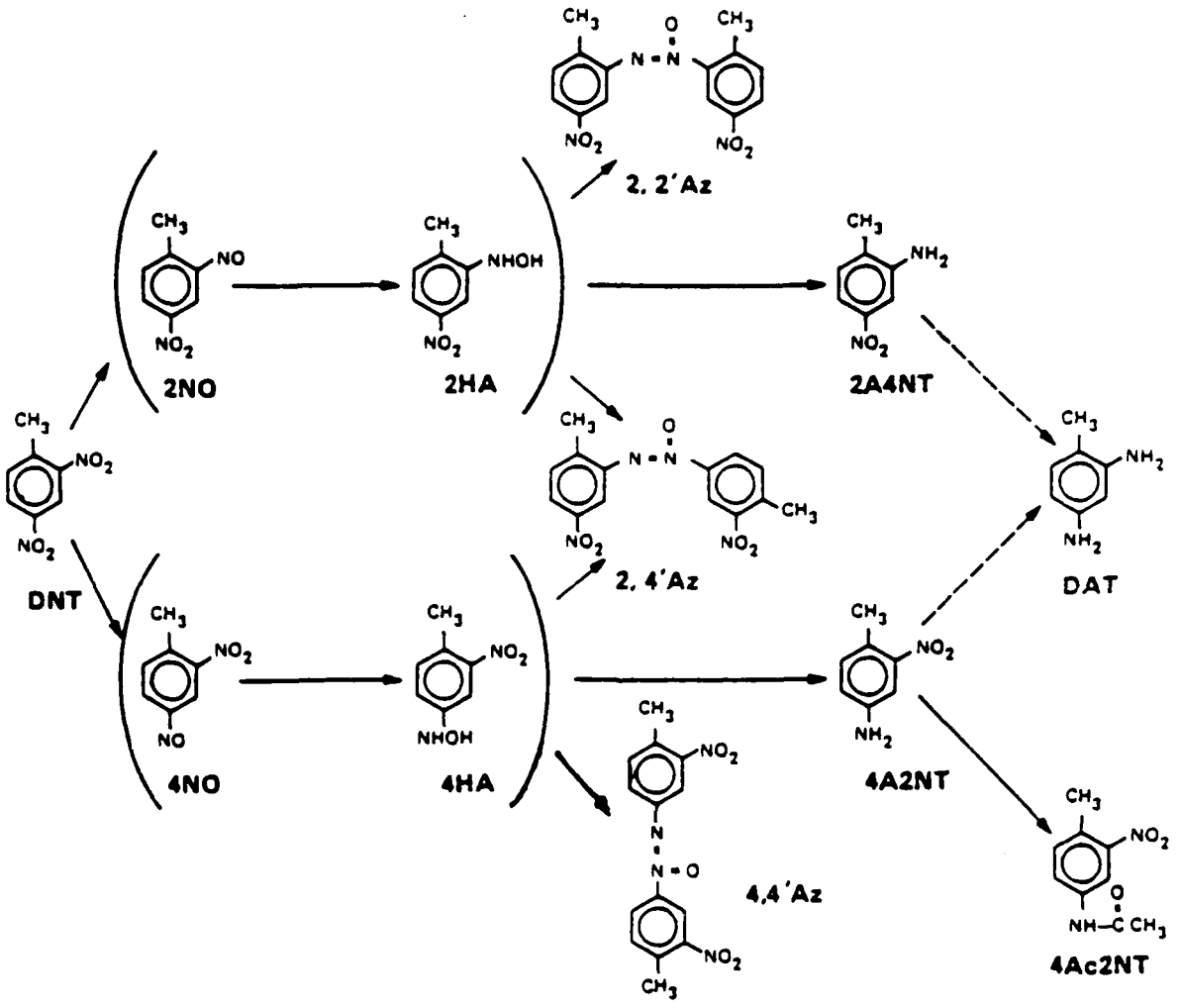


Figure 1. Proposed pathways for the biotransformation of 2,4-DNT. The nitroso and hydroxylamino intermediates were not detected by thin-layer chromatography or gas chromatography/mass spectrometry, but they are included in parentheses as hypothetical intermediates. The potential formation of 2,4-diaminitrotoluene is indicated by dashed arrows. From McCormick et al. 1978.

DNT = 2,4-dinitrotoluene
2NO = 2-nitroso-4-nitrotoluene
4NO = 4-nitroso-2-nitrotoluene
2HA = 2-hydroxylamino-4-nitrotoluene
4HA = 4-hydroxylamino-2-nitrotoluene
2,2'AZ = 4,4'-dinitro-2,2'-azoxytoluene
2,4'AZ = 4,2'-dinitro-2,4'-azoxytoluene
4,4'AZ = 2,2'-dinitro-4,4'-azoxytoluene
2A4NT = 2-amino-4-nitrotoluene
4A2NT = 4-amino-2-nitrotoluene
DAT = diaminotoluene
4Ac2NT = 4-acetamido-2-nitrotoluene

4-methyl-3-nitroaniline. Respiration rates using Warburg analysis were found to be inhibited in municipal sewage sludge at all test concentrations, i.e., 10 to 200 mg/L 2,4-DNT. In contrast, respiration rates showed a strange response in industrial sludge, with no appreciable inhibition at 100 mg/L 2,4-DNT and respiratory stimulation at 200 mg/L 2,4-DNT.

With respect to 2,6-DNT, the 50 mg/L concentration was reduced to 25 mg/L between day 2 and day 7 following exposure to industrial sewage sludge, with base-neutral-extracted samples showing a by-product at 7 days but not at 2 days, and with acid-extracted samples indicating no by-product formation. The transformation product identified at 7 days was 2-methyl-5-nitroamine. Respiratory rates in industrial sludge were unaffected at concentrations of 50 to 200 mg/L 2,6-DNT, but rates in municipal sludge respiration was inhibited at and above 50 mg/L (Davis et al. 1981).

Model waste stabilization ponds (aerobic) were used in an attempt to obtain a mass balance for 2,6-DNT that included biodegradation, volatilization, sedimentation, bioconcentration, and residual concentrations in the water column and effluent (Davis et al. 1983). Eighty applications of 1 mg/L were made in 40 days, and quantification and identification of the compound were accomplished by GC/MS. It was estimated that 92.5 percent of the 2,6-DNT had been biodegraded over the entire testing period. When adjusted for volatilization, the percent biodegradation was reduced slightly to 92.2 percent.

Bailey (1982) reported that the rate of loss of 2,4-DNT from aquatic model ecosystems was proportional to the concentrations of the chemical in the test system, although after an apparent induction period, the slope of the lines was similar for all concentrations tested. Rate of loss of 2,4-DNT was reduced in battery jars that had been autoclaved and contained no live biota.

2.2.3 Physical Degradation

In a study by Andrews and Osmon (1976), 2,4-DNT and 2,6-DNT were found to be completely photolyzed in a static UV system containing 0.1 percent hydrogen peroxide (H_2O_2). Irradiation at 240 to 260 nm resulted in complete elimination of 100 ppm 2,4- or 2,6-DNT after 1 hr, with no evidence of conversion products in the photolysate.

The synergistic effect of UV radiation and H_2O_2 on the degradation of 2,4-DNT was also studied by Ho (1986), with analysis of degradation products performed by HPLC, GC, and GC/MS. Photolytic degradation of 204.9 ppm 2,4-DNT was incomplete after 17 hr in the absence of H_2O_2, but degradation was complete after 1.5 hr with a molar ratio of H_2O_2/DNT between 26 and 52. Relative levels of H_2O_2 above these ratios were found to retard degradation. Studies with aqueous 2,4-DNT solution and H_2O_2 alone, without UV irradiation, showed degradation to be very slow and incomplete. The major photolysis product found, amounting to about 90 percent of the total degradation products detected (excluding

unreacted 2,4-DNT), was 2,4-dinitrobenzoic acid (2,4DNBA). Degradation pathways were suggested based on intermediates identified after photooxidation: side-chain oxidation converts 2,4-DNT to 2,4DNBA, which undergoes decarboxylation, and then to 1,3-dinitrobenzene, which is then converted to di- and trihydroxynitrobenzene derivatives via hydroxylation of the benzene ring; benzene ring cleavage of these hydroxynitrobenzenes produces carboxylic acids and aldehydes; the lower-molecular-weight acids and aldehydes are subsequently converted by further photooxidation to carbon dioxide, water, and nitric acid.

Photolysis of 2,4-DNT was found to be similar to that of TNT, with the rate accelerating over time in distilled water, presumably due to the presence of activated transformation products. Photolysis rates were found to be faster in natural waters than in distilled water (Spanggord et al. 1980a). Photolysis half-lives in sunlight were estimated to be from 2.7 to 9.6 hr in natural water and 43 hr in air-saturated distilled water. Spanggord et al. (1980b) calculated the photolysis half-life of 2,4-DNT as a function of latitude and season (Table 3). No detailed studies were performed to determine the mechanism of DNT photolysis. However, Spanggord et al. speculate that it probably occurs by a triplet excited mechanism similar to that suggested for TNT.

TABLE 3. ANNUAL VARIATION OF PHOTOLYSIS HALF-LIFE[a] OF 2,4-DNT[b] IN SUNLIGHT IN DISTILLED WATER[c]

Season	2,4-DNT half-life (N latitude)		
	20°	40°	50°
Summer	1.8	1.8	1.9
Fall	2.3	3.6	5.4
Winter	2.8	6.0	11.5
Spring	1.9	2.2	2.5

a. Half-lives are in 24-hr days (10 hr sunlight).
b. 2,4-DNT = 2,4-dinitrotoluene.
c. Adapted from Spanggord et al. 1980b.

Burlinson and Glover (1977a, as reported in Spanggord et al. 1980a) found that 2,4-DNT was photolyzed more rapidly at higher pH, with 44 and 92 percent removal at a pH of 3.3 and 10.8, respectively, following 5 hr of UV irradiation. They also reported the identification of photolysis reaction products and their yield: 2,4-dinitrobenzaldehyde, 6 percent; 2-amino-4-nitrobenzaldehyde, 10 percent; 2,2'-carboxaldehyde-5,5'-dinitroazoxybenzene (sic), 3 percent; 2,4-dinitrobenzoic acid, 7 percent; 2-amino-4-nitrobenzoic acid, 16 percent; and 2,2'-carboxy-5,5'-dinitroazoxybenzene (sic), 10 percent. Similarly, Burlinson and Glover (1977b, as reported in Spanggord et al. 1980a) found that loss of 2,6-DNT due to photolysis was 4, 18, and 31 percent at a pH of 4.6, 6.7, and

10.8, respectively. By products identified were azoxy and azobenzene structures with methyl and nitro substituents and their derivatives. A 150 ppm solution of 2,6-DNT contained only 0.2 ppm following 5-day exposure to sunlight (Burlinson and Glover 1977c, as reported in Spanggord et al. 1980a).

Randall and Knopp (1980) found that wet oxidation carried out in an autoclave at 320 and 275°C destroyed 99.88 and 99.74 percent, respectively, of a 10 g/L suspension of 2,4-DNT within 1 hr. The solution from the 320°C oxidation was analyzed in an attempt to identify the breakdown compounds, but only formic acid and acetic acid were positively identified, occurring in concentrations of 134 and 213 mg/L, respectively.

Spanggord et al. (1980a) suggest that the volatilization rate of 2,4-DNT is limited by gas-phase mass transport resistance. They estimate a half-life for volatilization of 47 to 410 days for the 2,4-isomer and 9 to 140 days for the 2,6- isomer.

In the model waste stabilization pond study of Davis et al. (1983), it was estimated that only 0.3 percent of the total 2,6-DNT applied to the system (2 mg/L/day for 40 days) was lost by volatilization. A half-life for vaporization of 200 hr was measured. Neither 2,4- or 2,6-DNT is expected to hydrolyze under environmental conditions (Spanggord et al. 1980a).

2.2.4 Sediment Adsorption

Very few data exist describing soil or sediment adsorption of 2,4- or 2,6-DNT. Spanggord et al. (1980b) estimate an adsorption partition coefficient (K_{OC}) of 90 for 2,4-DNT and 100 for 2,6-DNT, and they suggest that adsorption isotherm experiments be carried out to determine whether sediment adsorption of these DNT isomers is a significant environmental fate.

Munition wastes from Volunteer AAP were found to be deposited on sediment, with a concentration gradient evident (Sullivan et al. 1977). TNT residues in sediment were 3.1 mg/kg (dry weight) at the discharge point and 0.32 mg/kg at a point 0.5 miles into the bay. These residues dropped to <0.1 mg/kg from this point to the mouth of the bay (1.77 miles). Measurements of DNT in sediment were not reported.

Davis et al. (1983) reported that 3.6 percent of the total of 80 mg of 2,6-DNT added to a model waste stabilization pond was lost due to sedimentation. The pond sediments were principally made up of algal biomass.

2.3 SUMMARY

Biological degradation of 2,4- and 2,6-DNT occurs readily in anaerobic systems in the presence of an exogenous carbon source.

Aromatic diamines, aminonitrotoluenes, and nitrosonitrotoluenes have been detected in fermentor broth. Biodegradation of DNT is reduced in successive subcultures indicating that toxic effects from the isomers may be inhibiting degradation. It appears that the 4-nitro group is the most readily reduced, with almost all of it reduced before reduction of the 2-nitro group begins.

Both 2,4- and 2,6-DNT are completely photolyzed by UV light in the presence of hydrogen peroxide. The major photolysis product of 2,4-DNT is 2,4-dinitrobenzoic acid, with the ultimate products carbon dioxide, water, and nitric acid. Photolysis rates for 2,4-DNT are faster in natural water than in distilled water, and rate of loss is reduced in autoclaved water samples which contain no biota. Very few data exist describing soil or sediment absorption of either DNT isomer.

3. AQUATIC TOXICOLOGY

3.1 ACUTE TOXICITY TO ANIMALS

Summaries of the acute toxicity of 2,4-DNT and 2,6-DNT to freshwater aquatic life are presented in Tables 4 and 5, respectively. Forty-eight hour EC_{50} values for 2,4-DNT range from 26.2 to 47.5 mg/L for daphnids. Freshwater fish appear to be more sensitive to 2,4-DNT than invertebrates, with static 96-hr LC_{50} values of 13.5, 13.6, 24.8, and 32.8 mg/L for bluegills, rainbow trout, channel catfish, and fathead minnows, respectively. Only a few studies on the acute toxicity of 2,6-DNT are available, but from these data it appears that the 2,6-isomer is about twice as toxic to aquatic organisms as the 2,4- isomer. No studies were found that document the acute toxicity of either the 2,4- or the 2,6- isomer in saltwater organisms.

3.1.1 Aquatic Invertebrates

Short-term toxicity tests for 2,4-DNT with Daphnia magna (Adema et al. 1983) resulted in a 48-hr EC_{50} somewhat lower than reported by other authors (Table 4). A value of >10 and <16 mg/L was reported, with a "no observed effects concentration" (NOEC) of 10 mg/L. Test conditions for the studies were not given in the paper, and so the data are omitted from Table 4. Bringmann and Kuhn (1977) report a 24-hr LC_0 for Daphnia magna of 11 mg/L 2,6-DNT and an LC_{100} of 25 mg/L 2,6-DNT.

Liu et al. (1983) studied the effects of photolysis on the toxicity of 2,4-DNT to Daphnia magna. The 48-hr LC_{50} of the aqueous and benzene-extracted fractions of a 50-percent-photolyzed solution of 2,4-DNT was found to be greater than 50 mg/L for each fraction tested.

Using static toxicity tests with D. magna, Randall and Knopp (1980) found that the toxicity of a wet oxidized solution of 2,4-DNT (with greater than 99 percent removal of DNT) measured as the 48-hr EC_{50} was reduced by a factor of 15 when compared with an unoxidized solution of the same initial concentration (10 g/L). The 48-hr EC_{50} was 26.2 mg/L for the unoxidized solution.

3.1.2 Fish

Static and flow-through acute toxicity tests for 2,4-DNT were performed by Liu et al. (1983) using four species of fish (Table 4). They found that static LC_{50}s were lower than flow-through LC_{50}s, with the difference statistically significant ($p = 0.05$) for the fathead minnow (Pimephales promelas) and the channel catfish (Ictalurus punctatus); however, the authors felt that these differences were not due to the greater toxicity of degradation products, and that they were not environmentally significant. With all the species tested, including the invertebrates, the incipient LC_{50}s were significantly ($p < 0.05$) lower than the flow-through 96-hr LC_{50}s.

TABLE 4. ACUTE TESTS FOR IMMOBILIZATION[a] OR MORTALITY[b] OF AQUATIC SPECIES FOLLOWING EXPOSURE TO 2,4-DNT[c]

Test Species	Test Method	Test Duration	LC_{50}/EC_{50} (mg/L)	Reference
Arthropoda				
Crustacea				
Daphnidae				
Daphnia magna[a,d]	S[e]	48 h	35.0	Pearson et al. 1977
Daphnia magna[a,d]	S	48 h	47.5 (29.5-99.7)[f]	Liu et al. 1983
Daphnia magna[a,d]	S	48 h	38.3 (33.6-43.8)	Liu et al. 1983
Daphnia magna[a,d]	S	48 h	26.2	Randall and Knopp 1980
Daphnia magna[a]	S	24 h	22	Bringmann and Kuhn 1977
Daphnia magna[a,d]	FT[g]	48 h	30.4	Liu et al. 1983
Daphnia magna[a,d]	Incip[h]	14 d	4.1	Liu et al. 1983
Gammaridae				
Hyallela aztecta[a,i]	S	48 h	>83.2	Liu et al. 1983
Insecta				
Chironomidae				
Tanytarsus dissimilis[a,j]	S	48 h	22.5	Liu et al. 1983
Annelida				
Oligochaeta				
Lumbriculidae				
Lumbriculus variegatus[a,i]	S	48 h	>83.2	Liu et al. 1983
Lumbriculus variegatus[a,i]	FT	48 h	80.9	Liu et al. 1983
Lumbriculus variegatus[a,i]	Incip	14 d	30.4	Liu et al. 1983
Chordata				
Osteichthyes				
Centrarchidae				
Lepomis macrochirus[b,k]	S	96 h	13.5 (12.1-15.1)	Liu et al. 1983
Lepomis macrochirus[b,k]	FT	96 h	16.0	Liu et al. 1983
Lepomis macrochirus[b,k]	Incip	14 d	9.2	Liu et al. 1983

TABLE 4 (CONTINUED)

Test Species	Test Method	Test Duration	LC_{50}/EC_{50} (mg/L)	Reference
Salmonidae				
Salmo gairdneri[b,k]	S	96 h	13.6 (12.2-15.2)	Liu et al. 1983
Salmo gairdneri[b,k]	FT	96 h	13.9	Liu et al. 1983
Salmo gairdneri[b,k]	Incip	14 d	6.3	Liu et al. 1983
Ictaluridae				
Ictalurus punctatus[b,k,l]	S	96 h	24.8 (21.0-29.3)	Liu et al. 1983
Ictalurus punctatus[b,k]	FT	96 h	32.0	Liu et al. 1983
Ictalurus punctatus[b,k]	Incip	14 d	16.4	Liu et al. 1983
Poeciliidae				
Poecilia reticulata[b]	S	96 h	25	Adema et al. 1983
Cyprinidae				
Pimephales promelas[b,m]	S	96 h	32.8 (27.3-38.0)	Liu et al. 1983
Pimephales promelas[b,m]	S	96 h	28.5 (26.3-32.5	Liu et al. 1983
Pimephales promelas[b]	S	96 h	32.5	Pearson et al. 1977
Pimephales promelas[b,m]	FT	96 h	36.1	Liu et al. 1983
Pimephales promelas[b,m]	Incip	14 d	26.0	Liu et al. 1983

a. Immobilization tests were designed to give EC_{50} values.
b. Mortality tests were designed to give LC_{50} values.
c. 2,4-DNT = 2,4-dinitrotoluene.
d. Test animals were first instars at start of test (<12 hr old).
e. S = static test.
f. All values in parentheses represent the 95% confidence interval.
g. FT = flow-through test with measured concentrations.
h. Incipient LC_{50} values are those which cause 50% mortality after an extended exposure period.
i. Test animals were of unknown age.
j. Test animals were second or third instars at start of test.
k. Test animals were juveniles.
l. Test solutions aerated.
m. Test animals were 90-day-old juveniles.

TABLE 5. ACUTE TESTS FOR IMMOBILIZATION[a] OR MORTALITY[b] OF AQUATIC SPECIES FOLLOWING EXPOSURE TO 2,6-DNT[c]

Test Species	Test Method	Test Duration	LC_{50}/EC_{50} (mg/L)	Reference
Arthropoda				
Crustacea				
Daphnidae				
Daphnia magna[a,d]	S[e]	24 h	14	Bringmann and Kuhn 1977
Daphnia magna[a,d]	S	48 h	21.8 (19.3-24.6)[f]	Liu et al. 1983
Chordata				
Osteichthyes				
Cyprinidae				
Pimephales promelas[b,g]	S	96 h	18.5 (17.2-20.2)[f]	Liu et al. 1983

a. Immobilization tests were designed to give EC_{50} values.
b. Mortality tests were designed to give LC_{50} values.
c. 2,6-DNT = 2,6-dinitrotoluene.
d. Test animals were first instars at start of test (<12 hr old).
e. S = static test.
f. All values in parentheses represent the 95% confidence interval.
g. Test animals were 90-day-old juveniles.

A 96-hr LC_{50} value of 16 mg/L for bluegill sunfish (Lepomis macrochirus) was reported for an unspecified isomer of DNT (Burton 1972).

Liu et al. (1983) studied the effects of photolysis on the toxicity of 2,4-DNT to fathead minnows. A photolyzed solution of 2,4-DNT was prepared by exposure to filtered UV irradiation (simulated sunlight) in a batch reactor. A starting concentration of 150 mg/L 2,4-DNT was reduced to 75 mg/L after 50 percent photolysis and to less than 0.05 mg/L after complete photolysis. Estimates of the 96-hr LC_{50} for 0, 50, and 100 percent photolysis were 31.4, >35.0, and 64.8 mg/L, respectively, indicating that exposure to simulated sunlight reduced DNT toxicity. A two-fold reduction in the toxicity of the 2,4-DNT solution was accomplished by complete photolysis (statistically significant at $p \leq 0.05$). The toxicities of the aqueous and benzene-extractable fractions of a 50-percent-photolyzed solution of 2,4-DNT were found to be similar, with a 96-hr LC_{50} for fathead minnows of 35 and 22 mg/L, respectively.

The effects of water quality on the acute toxicity of 2,4-DNT to bluegill sunfish were also studied by Liu et al. (1983), and the results of their study are presented in Table 6. Temperature variations had the most pronounced effect on toxicity, with 2,4-DNT being two and three times as toxic at 20 and 25°C, respectively, relative to 17°C. 2,4-DNT was also slightly less toxic in soft water (47 mg/L $CaCO_3$) at a pH of 7 than it was at a pH of 6 or 8.

TABLE 6. ACUTE TOXICITY OF 2,4-DNT[a] TO BLUEGILL (Lepomis macrochirus) UNDER VARYING CONDITIONS OF WATER QUALITY DURING STATIC TOXICITY TESTS[b]

Water Quality Parameter	Desired Level	Actual Level	96-hr LC_{50} (mg/L)
Hardness[c] (mg/L $CaCO_3$)	40	47.0	12.8 (11.5-14.0)[d]
	100	109.0	18.8 (17.1-20.6)
	250	251.0	16.4 (14.8-17.9)
Temperature[e] (°C)	15	17.3	24.0 (18.0-32.0)
	20	20.8	12.8 (11.5-14.0)
	25	24.5	7.8 (7.2-8.5)
pH[f]	6	6.3	8.4 (7.0-10.0)
	7	7.1	12.8 (11.5-14.0)
	8	8.0	9.4 (8.7-10.4)

a. 2,4-DNT = 2,4-dinitrotoluene.
b. Adapted from Liu et al. 1983.
c. Nominal temperature and pH were 20°C and 7.0, respectively.
d. Values in parentheses represent 95% confidence intervals.
e. Nominal pH and hardness were 7.0 and 40 mg/L $CaCO_3$, respectively.
f. Nominal temperature and hardness were 20°C and 40 mg/L $CaCO_3$, respectively.

3.2 CHRONIC TOXICITY TO ANIMALS

No studies documenting the chronic toxicity of 2,6-DNT to aquatic organisms or the chronic toxicity of either the 2,4- or the 2,6- isomer to saltwater organisms were found.

3.2.1 Aquatic Invertebrates

Two-week testing with Daphnia magna, 4-week testing with Poecillia reticulata, and 6-week testing with Jordanella floridae (Adema et al. 1983) resulted in an NOEC of 0.32 to 1.0, 1.8 to 3.2, and 1.0 mg/L 2,4-DNT, respectively. The experimental conditions were not given in the report, although the authors specify that they followed the Organization for Economic Cooperation and Development (OECD) guidelines (OECD 1981), which recommend semi-static or flow-through conditions for prolonged toxicity tests with daphnids and fish.

Using an aquatic model ecosystem consisting of 3-L battery jars, sand as a substrate, and Lumbriculus variegatus, Daphnia magna, and Selenastrum capricornutum as the biological components, Bailey (1982) studied the toxic effects of 2,4-DNT using a 21-day exposure period. The total number of daphnids present was severely reduced at concentrations of 1.0 to 56.0 mg/L, with only 0 to 5 percent of adults surviving. In the two test jars with 0.6 mg/L 2,4-DNT, one population of daphnids increased above that seen in controls, and the other decreased to 50 to 60 percent of controls. Reproduction of daphnids was reduced, with none produced in the jars containing 5.6 to 56.0 mg/L, and only a few broods occurring in the 1.0 mg/L jar, all of which died within 11 days after initiation of the exposure. The total number of lumbriculid worms present after a 21-day exposure was slightly reduced from controls at concentrations of 0.6, 1.0, 5.6, and 10.0 mg/L 2,4-DNT, and reduced by about 50 percent at a concentration of 56 mg/L.

Bailey et al. (1984) also reported a significant ($p < 0.05$) reduction in number of young produced per parthenogenic female daphnid (Daphnia magna) after 21 days at a concentration of 0.40 mg/L 2,4-DNT, although time to first brood did not seem to be affected by DNT. Concentrations tested were 0.07, 0.19, 0.40, 1.02, and 1.78 mg/L 2,4-DNT. Average length of daphnids after 28 days was significantly ($p < 0.05$) reduced at concentrations of 1.02 mg/L 2,4-DNT. No dose-related mortalities were reported for concentrations up to 1.78 mg/L 2,4-DNT. Some question arises as to the validity of the tests performed by Bailey et al. (1984), since the reproductive capacity of the daphnid populations studied as controls was below that currently required by aquatic toxicity testing standards (OECD 1982; USEPA 1985). The USEPA (1985) standards require a minimum of 60 young produced per female in 21 days, and the Bailey et al. controls had an average of 39.9 young produced per female; another requirement is that adults in the culture produce young before day 12, and the Bailey et al. (1984) control cultures did not produce young until 12.3 to 15.2 days after initiation of the test.

3.2.2 Fish

Juvenile bluegill sunfish were exposed to sublethal concentrations of 2,4-DNT for 8 weeks under flow-through bioassay conditions (Hartley 1981). Two exposure series were used, with concentrations of 0.5, 2.0, 5.0, and 8.0 mg/L 2,4-DNT for the first series and 0.05, 0.50, 1.0, 2.0, and 4.0 mg/L for the second series. Subacute effects studied included histological and physiological responses. Using a first-order growth model, Hartley found significant decreases in growth rates as concentration increased up to 4.0 mg/L, with no growth at 5.0 and 8.0 mg/L. A subacute growth response threshold for 2,4-DNT of approximately 0.05 mg/L was estimated. Significant histopathological changes were found in liver, trunk kidney, spleen, lateral line, and gill of fish exposed to 0.5 to 8.0 mg/L. Hypertrophy of gill lamellae was observed in 20 percent of fish exposed to 0.5 and 2.0 mg/L after 42 to 56 days of exposure, with severe hyperplasia demonstrated in gills of fish at concentrations of 5.0 and 8.0 mg/L 2,4-DNT after 49 to 56 days. Large hemosiderin inclusions were found in the spleen of 40 percent of the fish after 45 days of exposure to 4.0 mg/L. Ten to 20 percent of fish exposed to 5.0 and 8.0 mg/L developed lipid accumulation with associated necrotic foci in the liver, atypical trunk renal tubules, and tubule necrosis after 49 days. After 56 days exposure to 5.0 mg/L, 20 percent of fish developed atypical neuromast cells and necrotic epithelium of the lateral line. Ataxic or irregular swimming observed at the higher 2,4-DNT exposures might be related to the lateral line lesions. No changes were found in the gut, pancreas, integument, heart, gonad, head kidney, or spinal cord of fish exposed to 2,4-DNT.

In early-life-stage studies, egg hatchability, fry survival and overall growth of rainbow trout (Salmo gairdneri) exposed to concentrations of 0.10, 0.23, 0.49, 1.08, and 2.05 mg/L 2,4-DNT for 60 days were analyzed by Bailey et al. (1984). Hatching success and fry survival were not affected at any of the concentrations tested after 60 days of exposure; however, the effects on growth showed a dose response at the three highest concentrations tested. Length was reduced 10.2, 20.0, and 23.2 percent and weight was reduced 26.6, 45.0, and 51.7 percent at concentrations of 0.49, 1.08, and 2.05 mg/L 2,4-DNT, respectively (significant at $p \leq 0.05$). A significant reduction ($p \leq 0.05$) in fry weight was seen at 0.10 mg/L, but not at 0.23 mg/L, possibly due to the relatively few fish exposed to the latter concentration. Similarly, hatching success, fry survival, and number of deformed rainbow trout fry were unaffected after 90 days at concentrations of 0.05, 0.12, 0.27, 0.56, 1.17, 2.26, or 4.02 mg/L 2,4-DNT. However, all of the fry tested at the highest concentration (4.02 mg/L) were unable to swim and remained on their sides on the bottom of the aquaria. In one of the two test series (Series A) run at 0.05 to 4.02 mg/L 2,4-DNT, average length and weight were significantly reduced ($p \leq 0.05$) from controls at all concentrations tested. However, reductions in length were only 5 to 9 percent for concentrations up to 0.56 mg/L. Weight reductions varied from 2 to 29 percent for concentrations of 0.27 to 1.17 mg/L 2,4-DNT. In Series A, survival rate in controls was 74 percent, slightly below the current acceptable USEPA standard of 80 percent (USEPA 1985). This may have affected the percentage of fish experiencing weight and length

reductions. In the other test series (Series B), with 82 percent survival in controls, length was reduced significantly ($p \leq 0.05$) at concentrations of 1.17 to 4.02 mg/L, and weight was reduced at concentrations of 2.26 and 4.02 mg/L. If only the data from the series with acceptable survival rates in the control group (Series B) are considered, an NOEC of 0.56 mg/L can be assumed.

Early-life-stage studies (30 days) with fathead minnows showed no effects of 2,4-DNT on egg hatchability, fry growth, and fry survival at concentrations of 1.0, 1.1, 2.0, or 3.1 mg/L (Bailey et al. 1984). Although no statistical analysis was performed, all three parameters studied were markedly reduced at a concentration of 6.8 mg/L 2,4-DNT when compared with controls.

A 6-month study with fathead minnows indicated a significant decrease ($p < 0.05$) in number of eggs hatched and in fry survival at 2.69 and 6.71 mg/L 2,4-DNT, with no significant effects seen at 1.31 mg/L or lower (Bailey et al. 1984). Effects on fry length were sporadic and transient at all concentrations studied (0.28, 0.62, 1.31, 2.69, and 6.71 mg/L 2,4-DNT), with the significant reductions seen at 30, 60, or 90 days reversed during the remainder of the study. Chronic exposure to 2,4-DNT significantly reduced ($p < 0.05$) egg production in surviving spawning pairs at a concentration of 0.62 mg/L and higher, with some effects (not statistically significant) seen at 0.28 mg/L.

The effect of 2,4-DNT on the F_1 generation of fathead minnows was also reported by Bailey et al. (1984) for concentrations of 0.28, 0.62, 1.31, 2.69, or 6.71 mg/L 2,4-DNT. In both of the test series (A and B), a small (<5 percent) but statistically significant reduction ($p < 0.05$) in egg hatching success occurred at 2.69 mg/L, with a statistically significant ($p < 0.05$) increase in fry deformities at concentrations of 0.28, 0.62, and 1.31 mg/L for Series A. Survival of fry reared to 60 days was significantly reduced ($p < 0.05$) at concentrations of 0.28 and 0.62 mg/L 2,4-DNT in Series A and at 1.31 mg/L in Series B, but not at 1.31 and 2.69 mg/L in Series A. In Series B, length of fry raised to 60 days was significantly reduced ($p < 0.05$) at 0.28 and 0.62 mg/L, but not at the higher concentrations tested. Significant weight reductions seen at 30 days in Series A at concentrations of 0.28, 0.62, and 2.69 mg/L were reversed at 60 days.

Bailey et al. (1984) estimated indices of total survivability and total productivity for fathead minnows and showed a definite dose response with both parameters decreasing as 2,4-DNT concentration increased.

3.3 TOXICITY TO MICROORGANISMS AND PLANTS

3.3.1 Bacteria and Protozoa

Bringmann and Kuhn (1980) report a toxicity threshold (the concentration that will produce 3 percent growth inhibition) for the bacterium

Pseudomonas putida grown for 16 hr in a culture solution containing 2,4- or 2,6-DNT. 2,4-DNT proved to be less toxic, with a toxicity threshold (TT) of 57 mg/L, compared with a TT of 26 mg/L for 2,6-DNT. Similar studies using holozoic and saprozoic protozoans and a TT based on 5 percent growth inhibition were reported by Bringmann and Kuhn (1978, 1981). The holozoic ciliate protozoan Uronema parduczi Chatton-Lwoff was the most sensitive to 2,4-DNT toxicity, with a TT of 0.55 mg/L, and the most resistant to 2,6-DNT, with a TT of 23 mg/L (Bringmann and Kuhn 1981). The holozoic flagellate Entosiphon sulcatum Stein, had a TT of 0.98 mg/L for 2,4-DNT and 11 mg/L for 2,6-DNT (Bringmann and Kuhn 1978). The saprozoic flagellate Chilomonas paramecium Ehrenberg was the most resistant of the protozoans, with a TT of 13 mg/L for 2,4-DNT, and >20 mg/L for 2,6-DNT (Bringmann and Kuhn 1981).

The effective concentration causing a 50 percent reduction in bacterial photoluminescence (EC_{50}) was reported for five isomers of DNT using the photobacterium Beneckea harveyi (Shiotsuka et al. 1980). The 2,5- isomer was the most toxic and the 2,6- isomer was the least toxic, with EC_{50}s of 3.45 and 20.25 mg/L, respectively. 2,4-DNT exhibited intermediate toxicity, with an EC_{50} value of 8.26 mg/L.

3.3.2 Aquatic Algae

Adema et al. (1983) performed 4-day toxicity tests with Scenedesmus pannonicus, using a cell multiplication index to indicate growth. They report a 96-hr EC_{50} for 2,4-DNT of 2.3 mg/L and an NOEC of 0.32 mg/L. Bringmann and Kuhn (1980) report the concentration that will produce 3 percent growth inhibition in S. quadricauda after a 7-day incubation period to be 2.7 mg/L for 2,4-DNT and 12 mg/L for 2,6-DNT. No other algal toxicity studies using 2,6-DNT were found.

In the aquatic model ecosystem used by Bailey (1982), algal growth in 3-L battery jars, as measured by density, was inhibited by concentrations of 1.0 to 56.0 mg/L 2,4-DNT. Following addition of the 2,4-DNT, algal populations at 5.6, 10.0, and 56.0 mg/L declined immediately, while populations at 0.6 and 1.0 mg/L continued to increase, although at a slower rate than controls, declining several days after introduction of toxicant. This presumably was due to grazing by the daphnids in the ecosystem. Algal population growth at all concentrations tested began to increase dramatically toward the end of the experiment, probably because of decline in feeding daphnid populations discussed in Sect. 3.2.1.

Liu et al. (1983) studied the toxic effects of 2,4-DNT on the growth of four algal species: Selenastrum capricornutum (green alga), Microcystis aeruginosa (bluegreen alga), Anabaena flos-aquae (bluegreen alga), and Navicula pelliculosa (diatom). Table 7 lists the results of their 14-day study. There was >98 percent growth inhibition in S. capricornutum after four days at concentrations of 4.7 mg/L and higher, and >99 percent inhibition after 14 days at concentrations of 9.4 mg/L and higher. A decrease in growth inhibition was seen between 4 and 14 days at a concentration of 0.9 and 4.7 mg/L, indicating a partial

TABLE 7. EFFECT OF 2,4-DNT[a] ON ALGAL POPULATION GROWTH UNDER STATIC CONDITIONS[b]

Algal Species	Concentration[c] (mg/L)	Percentage of Effect[d] 4 Days	14 Days
Selenastrum capricornutum	0.9	-37.4[e]	-13.5
	4.7	-98.1[e]	-42.5[e]
	9.4	-99.5[e]	-99.5[e]
	23.6	-99.6[e]	-99.8[e]
	47.2	-99.5[e]	-99.8[e]
	94.4	-99.6[e]	-99.8[e]
Microcystis aeruginosa	0.05	0.0	-4.2
	0.1	-20.2	-13.8
	0.5	-57.2[e]	-33.4[e]
	1.0	-65.8[e]	-48.5[e]
	5.0	-81.3[e]	-97.8[e]
	10.0	-80.5[e]	-99.5[e]
Anabaena flos-aquae	0.9	[f]	-23.4[e]
	4.7	[f]	-17.3
	9.4	[f]	-24.8[e]
	23.6	[f]	-98.7[e]
	47.2	[f]	-99.2[e]
	94.4	[f]	-99.3[e]
Navicula pelliculosa	1.0	[f]	+5.1
	4.9	[f]	+5.1
	9.8	[f]	-91.0[e]
	24.5	[f]	-97.8[e]
	49.0	[f]	-97.8[e]
	98.0	[f]	-97.5[e]

a. 2,4-DNT = dinitrotoluene.
b. Adapted from Liu et al. 1983.
c. These concentrations are probably higher than actual test concentrations, as the tests were run in the light and 2,4-DNT was probably actively photolyzed.
d. Relative to controls; a negative sign indicates inhibition, a positive sign denotes stimulation.
e. Statistically different from controls at $p = 0.05$.
f. Cell counts performed only on day 14.

recovery of the algal population. A. flos-aquae and N. pelliculosa were tested at similar concentrations, but cell counts were performed only on day 14. They appear to be slightly more tolerant to 2,4-DNT toxicity, with >97 percent growth inhibition at 23.6 and 24.5 mg/L, respectively. M. aeruginosa was tested at lower concentrations than the other algae, but reacted similarly to S. capricornutum, exhibiting recovery between day 4 and day 14 at concentrations up to 1.0 mg/L and >99 percent growth inhibiton at day 14 at 10.0 mg/L. Growth inhibition was significantly ($p = 0.05$) reduced (57 percent) after 4 days at 0.5 mg/L. It should be noted that the plateau phase of algal growth was not given, and a question of nutrient limitation after 14 days might be raised.

3.3.3 Flowering Plants

The duckweed, Lemna perpusilla Torr., was grown in acidic (pH 6.3) and basic (pH 8.5) media containing either 2,4-DNT or its metabolite 4A2NT (Schott and Worthley 1974). Toxicity was expressed as a decrease in number of fronds counted, or death, after 11 days exposure. In both acidic and basic media, colony death was seen at 5.0 mg/L 2,4-DNT and higher, growth was depressed at concentrations of 0.5 to 1.0 mg/L, and a no-effect level was seen at 0.1 mg/L 2,4-DNT. At the lower pH (6.3), 4A2NT had no effect at 10 mg/L and lower, with colony growth depressed at 50 mg/L and death seen at 100 mg/L. In the pH 8.5 media, colony growth was depressed at 10 to 100 mg/L 4A2NT, with no death of colonies reported. The no-effect level for 4A2NT was between concentrations of 1.0 and 10.0 mg/L. No information was found for the 2,6- isomer.

3.4 BIOACCUMULATION

Bluegill sunfish were exposed to 2.91 ± 0.18 mg/L ^{14}C ring-labelled 2,4-DNT for 14 days (Hartley 1981). The data were fitted to zero-order kinetic models; and uptake rates, loss rates, and bioconcentration factors (BCF) were reported for seven tissues (Table 8). High uptake and clearing (loss) rates were seen for brain and kidney, corresponding with relatively high BCFs. As seen in Table 8, bioconcentration levels below 100 were observed for all tissues but brain, with the highest levels seen in kidney and liver, and lower levels observed in whole body and striated muscle. Rapid absorption of 2,4-DNT occurred (24 to 72 hr), with total elimination of 2,4-DNT or its metabolites occurring 24 to 72 hr after fish were placed in a 2,4-DNT-free environment.

Four-day bioaccumulation studies using ^{14}C ring-labelled 2,4-DNT were reported by Liu et al. (1983). The nominal exposure concentration of 2,4-DNT was 1 mg/L. The four-day BCFs for S. capricornutum, D. magna, and Lumbriculus variegatus were 2507, 13, and 58, respectively. The relatively high value for the green alga S. capricornutum might have been due to adsorption of 2,4-DNT on the cell walls (Liu et al. 1983). The 4-day BCF for bluegill sunfish was 78 for viscera and 4 for muscle. In bluegills, rapid clearance of 2,4-DNT or its metabolites occurred, with none remaining after 4 days. The BCF of a simulated condensate wastewater containing 43 percent 2,4-DNT (1.3 mg/L) was almost identical to the BCF for 2,4-DNT alone for the four species studied.

TABLE 8. UPTAKE, BIOCONCENTRATION, AND LOSS OF ^{14}C-LABELLED 2,4-DNT[a] IN Lepomis macrochirus TISSUE AFTER TWO WEEKS OF EXPOSURE[b]

Tissue	Zero-Order Uptake Rate[c]	Bioconcentration Factor	Zero-Order Loss Rate[d]
Whole body	15.36 ± 1.40	24.8	24.94 ± 3.78
Brain	43.76 ± 9.52	102.8	77.09 ± 5.07
Kidney	23.64 ± 1.93	48.25	31.30 ± 4.73
Stomach/ intestine	18.70 ± 4.35	29.2	19.96 ± 4.42
Gill	16.40 ± 3.02	23.62	19.62 ± 4.71
Liver	14.22 ± 2.11	29.41	24.56 ± 1.72
Striated Muscle	6.02 ± 0.586	10.59	9.53 ± 1.43

a. 2,4-DNT = 2,4-dinitrotoluene.
b. Adapted from Hartley 1981.
c. Micrograms 2,4-DNT per gram tissue per day uptake ± standard deviation.
d. Micrograms 2,4-DNT per gram tissue per day loss ± standard deviation.

Using the equation of Veith et al. (1978): log BCF = 0.76 log P - 0.23, and a calculated log P (octanol/water partition coefficient) value of 2.28, Liu et al. (1983) estimated a bioconcentration factor of 31.83 for the six DNT isomers. Based on measured values reported in the literature, this probably represents a conservative estimate. Ellis et al. (1979) report an estimated BCF of 18.8 for 2,4-DNT.

Using a model waste stabilization pond and a total input of 80 mg 2,6-DNT over a 40-day time period, Davis et al. (1983) analyzed the amount of DNT present on centrifuged and decanted algae and estimated a BCF of 5225 for the 2,6- isomer. It is possible that this high value was due to adsorption of DNT on the cell walls, as hypothesized by Liu et al. (1983) in their algal studies.

3.5 FIELD STUDIES

In a field study conducted at the Volunteer AAP, Sullivan et al. (1977) studied the effects of effluent releases on periphyton, phytoplankton, and benthic communities during a 3-month period in Waconda Bay. One month prior to the initiation of the survey in June, 1.3 million pounds of TNT was produced at the plant; however, the plant was closed down during the period of the study. In June a high concentration of 172 ppb 2,4-DNT, 144 ppb 2,6-DNT, and 71 ppb TNT were reported at the point of effluent discharge (Station A), with nondetectable levels at the mouth of the bay (2 miles from Station A) and in two nearby reference bays. There were no consistent patterns in concentration of the three compounds relative to distance from the discharge.

During the August survey, munitions-related residues were somewhat lower at Station A, with highs of 71, 116, and 70 ppb measured for 2,4-DNT, 2,6-DNT, and TNT, respectively.

Sullivan et al. (1977) reported that diatom populations collected on artificial substrate were reduced 84 to 95 percent in June and 70 to 71 percent 4 weeks later at Station A, when compared with reference bay populations. In terms of total numbers of species, Station A was also depressed, containing only 14 species of diatoms in June and 24 species in July, compared with 28 to 37 species at the reference stations in June and 31 to 37 species in July. They found that sensitive species were eliminated, normal colonizing species had their reproductive rates sharply reduced, and tolerant species became more common. The authors attribute these population changes to elevated munitions-derived residues and nitrate-nitrogen levels at Station A. Similar results were reported for the August sampling period, although a recovery trend was noted. Diatoms collected from natural substrate at the same site showed higher species diversity, but lower cell density per square millimeter.

A total of 70 phytoplankton genera were identified by Sullivan et al. (1977) during their sampling periods. They were unable to find any significant trends in the number of species recorded per station or in biotic similarity, and they failed to find any evidence of munitions toxicity. They did report biostimulation of phytoplankton populations residing at the end of Waconda Bay away from the point of discharge.

Macroinvertebrate populations studied by Sullivan et al. (1977) included snails, clams, arthropods, annelids, planarians, and coelenterates. Chironomid larvae comprised nearly 80 percent of the total number of organisms collected on artificial substrate. The data indicate that the immediate area where Volunteer AAP effluents enter Waconda Bay represents a zone of severe inhibition of population size and diversity. Strong evidence of residual sediment toxicity was apparent when comparing natural-substrate populations with artificial-substrate populations during the two study periods. Within the area between sampling stations A and B, a definite biostimulatory effect was noticed. Since concentrations of munitions compounds between these stations were similar, the authors state that it is unlikely that the toxicity seen at Station A was due entirely to the munitions compounds. Precise cause-and-effect relationships between population dynamics and concentrations of munitions compounds were impossible to establish from this study. However, it was observed that when concentrations dropped below 20 ppb, no biologic effects were evident. At concentrations between 40 and 80 ppb, slight biostimulatory effects were seen.

3.6 SUMMARY

Freshwater fish appear to be somewhat more susceptible to 2,4-DNT toxicity than freshwater invertebrates, having ranges of LC_{50} values from 13.5 to 32.8 mg/L 2,4-DNT in 96-hr static tests and 13.9 to 36.1 mg/L in 96-hr flow-through tests. The range of LC_{50} values for freshwater invertebrates was >10 to 83.2 mg/L 2,4-DNT. Incipient LC_{50} values (14-day) were lower than static or flow-through in all species studied, and they were lower for daphnids (4.1 mg/L) than for fish (6.3

and 9.2 mg/L for rainbow trout and sunfish, respectively). Studies with Daphnia magna indicate that 2,6-DNT is more toxic than 2,4-DNT, with LC_{50} values for 2,6-DNT about half those reported for 2,4-DNT. 2,6-DNT is also more toxic to freshwater fish, with an LC_{50} for the fathead minnow of 18.5 mg/L 2,6-DNT, compared with 28.5 to 32.8 mg/L 2,4-DNT. Photolysis, wet oxidation, temperature, and water hardness were found to affect 2,4-DNT acute toxicity.

Daphnid populations were severely affected during a 21-day exposure period at concentrations as low as 1.0 mg/L 2,4-DNT, with reproduction affected at 0.40 mg/L. Chronic exposure of bluegill sunfish to 2,4-DNT resulted in significant histopathological changes at a concentration of 0.5 mg/L. Hatching success and fry survival of rainbow trout were unaffected after 90 days at concentrations up to 2.26 mg/L, with behavioral effects seen in fry at 4.02 mg/L. However, 5 to 9 percent reductions in length and 12 to 20 percent reductions in weight were seen in one series of exposures for concentrations from 0.05 to 0.56 mg/L (control survival rates were low in this series). In the other test series, with 82 percent survival in controls, length was reduced significantly at concentrations from 1.17 to 4.02 mg/L, and weight was reduced at concentrations of 2.26 and 4.02 mg/L. If only the experiment with acceptable survival rates in controls is considered, an NOEC of 0.56 mg/L 2,4-DNT can be assumed. Early-life-stage studies (30-day) with fathead minnows showed no effects on egg hatchability, fry growth, or fry survival at concentrations of 2,4-DNT up to 3.1 mg/L. However, a 6-month study on fathead minnows indicated a significant decrease in number of eggs hatched and fry survival at 2.69 mg/L 2,4-DNT. Egg production was significantly reduced in fathead minnows at 0.62 mg/L; and a decrease in survival of fry, increase in fry deformities, and reduced length of fry were seen at 0.28 mg/L 2,4-DNT. No studies documenting the chronic toxicity of 2,6-DNT to freshwater organisms or the acute or chronic toxicity of 2,4- or 2,6-DNT to saltwater organisms were found.

Bacteria seem to be more tolerant to 2,4-DNT than to 2,6-DNT, with a toxicity threshold (TT) (that concentration producing a 3 percent reduction in growth) of 57 mg/L and 26 mg/L, respectively. Protozoans and flagellates appear to be more sensitive to 2,4-DNT than 2,6-DNT, with a TT of 0.55 to 13 mg/L and 11 to 23 mg/L, respectively. A 96-hr EC_{50} of 2.3 mg/L 2,4-DNT for the alga Scenedesmus pannonicus has been reported, with cell multiplication the endpoint. Algal growth has also been inhibited at concentrations of 0.5 mg/L 2,4-DNT. Toxicity of 2,4-DNT to flowering plants is seen by growth depression of Lemna perpusilla at a concentration of 0.5 mg/L. No information was found documenting the toxicity of 2,6-DNT to algae and plants.

Bioaccumulation factors for 2,4-DNT in striated muscle of bluegill sunfish are reported as 4 to 11, although values up to 100 have been reported in bluegill brain. A BCF of 13 has been reported for Daphnia magna. Relatively high BCFs in algae have been reported for both isomers (2507 and 5225 for 2,4- and 2,6-DNT, respectively), possibly due to adsorption onto the cell walls rather than bioaccumulation. A BCF of 18.8 has been estimated for 2,4-DNT, and a BCF of 31.8 has been calculated for all six isomers, based on the octanol/water partition coefficient.

4. MAMMALIAN TOXICOLOGY AND HUMAN HEALTH EFFECTS

4.1 PHARMACOKINETICS

Many studies exist that describe the pharmacokinetics of DNT in rats and mice, as well as other experimental animals. The current hypothesis regarding 2,4-DNT metabolism and excretion involves reduction by intestinal microflora, enterohepatic cycling, and sex-related differences in elimination of metabolites. Brief summaries of some of the in vivo and in vitro tests substantiating this theory are given below. Figure 2 shows the schematic representation of the metabolism and enterohepatic circulation of 2,4-DNT. In all of the radioactive tracer studies summarized in this report, a ^{14}C ring-labelled form of 2,4-DNT or 2,6-DNT was used.

4.1.1 Absorption

Following oral administration of 2,4-DNT to female Charles River CD rats, 80 to 90 percent of the administered dose was absorbed within 24 hr. Absorption of 2,6-DNT was slightly slower, with 60 percent absorbed in the same time period (Lee et al. 1975). Peak time for the absorption of radiolabelled 2,4-DNT from the small intestine was 6 hr in Wistar rats (Mori et al. 1978). Absorption was 90 to 100 percent following subchronic and chronic feeding (3, 9, or 20 months) of 2,4-DNT to male and female Charles River CD rats (Ellis et al. 1979). It appears that the 2,4-DNT metabolites that are excreted in the bile are subsequently reabsorbed from the intestines (Medinsky and Dent 1983).

Lee et al. (1978) studied the distribution and metabolism of 2,4-DNT in albino CD-1 and B6C3F1 mice. In contrast to the above studies, they reported that net absorption of 2,4-DNT in mice approximated 17 percent of the administered dose.

Estimates of 2,4- and 2,6-DNT uptake by inhalation from workroom air, absorption, and subsequent excretion in urine were made during work shifts of 11 employees in a DNT manufacturing plant (Levine et al. 1985). An excess of urinary 2,4-DNT metabolites was identified compared with the amount of 2,4-DNT measured in inspired air. The authors postulate that the unexplained quantities of 2,4-DNT found in urine may have been absorbed through the skin or through the gastrointestinal (GI) tract following accidental ingestion. Similar conclusions were drawn by Woolen et al. (1985) following a study of urinary excretion of 2,4-DNT metabolites from employees at an explosives factory; i.e., the levels of DNT metabolites appearing in the urine could not be correlated with the low levels of atmospheric DNT measured in the workroom. The authors suggest that skin absorption was the most likely alternate pathway of absorption. Their studies indicate that DNT is rapidly absorbed and metabolised following inhalation and/or skin absorption. The National Safety Council (NSC 1976) lists skin absorption of DNT as a route of exposure.

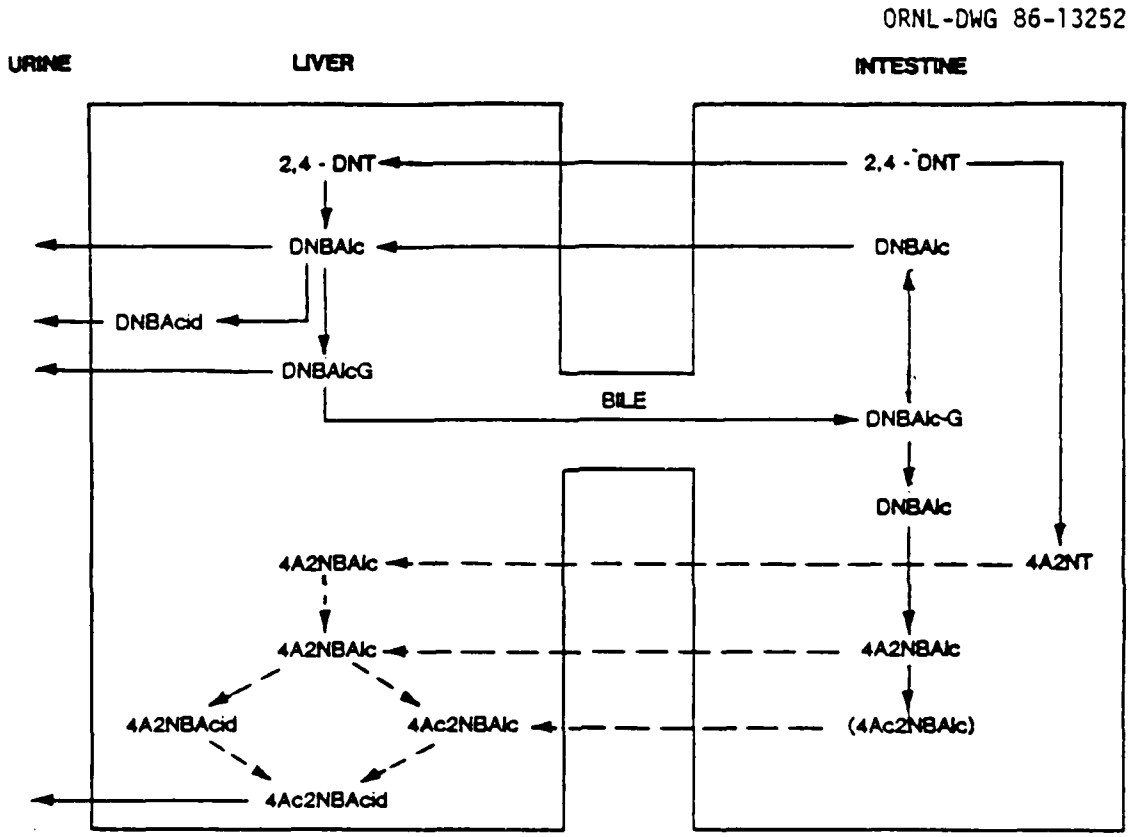


Figure 2. Proposed pathways for the metabolism and enterohepatic circulation of 2,4-DNT. Solid lines denote known pathways, and broken lines denote hypothetical pathways. Compounds are: DNBAlc = dinitrobenzyl alcohol, DNBAlcG = dinitrobenzyl alcohol glucuronide, DNBAcid = dinitrobenzoic acid, 4A2NT = 4-amino-2-nitrotoluene, 4A2NBAlc = 4-amino-2-nitrobenzyl alcohol, 4Ac2NBAlc = 4-acetyl-2-nitrobenzyl alcohol, 4A2NBAcid = 4-amino-2-nitrobenzoic acid. From Medinsky and Dent, 1983.

4.1.2 Distribution

Studies of the distribution of DNT in tissues indicate that small, but significant amounts of administered radiolabel are found in liver, blood, kidney, brain, and skeletal muscle, and that the liver is the major site of metabolism in rats (Lee et al. 1975; Mori et al. 1977; Rickert and Long 1980; Schut et al. 1982). Similar results were reported for mice, dogs, rabbits, and rhesus monkeys (Lee et al. 1978). Tissues in rats fed radiolabelled 2,4-DNT for 5 consecutive days contained 2 to 4.8 times the amount of radiolabel contained in tissues in rats fed a single dose of DNT (Lee et al. 1975). However, chronic feeding of labelled 2,4-DNT to male Wistar rats for 4 months resulted in less total uptake in tissues of a final single oral dose than was seen in untreated rats, indicating that the organs of treated rats were possibly saturated with 2,4-DNT or its metabolites (Mori et al. 1980).

Uptake of 2,4-DNT by rat and mouse liver and kidney is 2 to 10 times higher than by plasma or red blood cells, and about twice as high as by the lung, spleen, fat, and brain (Rickert and Long 1980; Schut et al. 1982). No significant difference between male and female rats was seen in the concentration or elimination of ^{14}C from plasma or in elimination from the liver (terminal half-lives of 30 and 41 hr for males and females, respectively) following administration of radiolabelled 2,4-DNT. However, from 2 to 72 hr after dosing, the concentration of ^{14}C was 2 times higher in livers of males than in livers of females (Rickert and Long 1980). Long and Rickert (1982) report the same results for uptake of radiolabelled 2,6-DNT in rat liver. In their earlier study, Rickert and Long (1980) reported that female rats retained 2,4-DNT or its metabolites in red blood cells for a longer period of time than male rats, with terminal half-lives of 123 hr for female rats and 27 hr for male rats. However, plasma levels of 2,4-DNT had similar terminal half-lives for male and female rats (2.2 and 2.5 hr, respectively), and were not detectable after 12 hr. Mori et al. (1978) reported a half-life for 2,4-DNT in blood (22 hr) of male Wistar rats dosed with ^{3}H-labelled 2,4-DNT, with blood levels of radioactivity peaking at 6 hr and gradually declining over the following 9 hr.

Peak levels of radioactivity in liver following administration of radiolabelled 2,4-DNT to male Wistar rats occurred at 6 hr, paralleling that found in blood (Mori et al. 1978), with radioactivity levels in stomach and small intestine rapidly declining at 6 hr.

Covalent binding of 2,4-DNT to hepatic macromolecules in bile-duct-cannulated Fischer 344 rats with bile removed was lower than in controls without bile removal, and was lower in female than in male rats (Medinsky and Dent 1983). Hepatic covalent binding was also found to be greater in male than in female Fischer 344 rats for both conventional animals and animals containing no intestinal microflora (axenic animals) and greater in conventional male than in axenic male rats. Conventional and axenic female rats showed similar hepatic binding of DNT (Rickert et al. 1981). Hepatic binding of 2,6-DNT in protein, RNA, and DNA is 2 to 5 times greater than that of 2,4-DNT, although half-lives for uptake and elimination are similar for both isomers. For both isomers, covalent

binding became significant only after the isomeric dinitrobenzyl alcohol glucuronide (DNBAlcG) had appeared in the small intestine. The concentration of the DNBAlcG declined in the intestine before peak covalent binding occurred in the liver (Rickert et al. 1983).

Swenberg et al. (1983) similarly reported sex differences in covalent binding of 2,6-DNT to hepatocyte DNA following a single oral dose of 35 mg/kg, with hepatocytes of female Fischer 344 rats showing slightly less binding than those of male rats. Considerably less 2,6-DNT was found bound to DNA in liver sinusoidal lining cells than to hepatocyte DNA of both sexes, with minimal DNA repair observed. Covalent binding of 2,6-DNT to hepatocyte DNA in axenic male rats was 10 percent of the amount bound to DNA of conventional rats. Male rats were given 35 mg/kg of radiolabelled 2,6-DNT for 1, 2, 3, or 4 weeks, and killed 7 days later. Covalently bound radioactivity was found only in hepatocellular DNA, with virtually none measured in sinusoidal lining cells, indicating a strong cell specificity for 2,6-DNT covalent binding.

Sulfotransferase inhibitors were used to determine whether sulfate ester formation is an important step in the in vivo bioactivation of 2,4- and 2,6-DNT to electrophilic species which covalently bind to DNA (Kedderis et al. 1984). Male Fischer 344 rats were administered pentachlorophenol (PCP) or 2,6-dichloro-4-nitrophenol (DCNP) and dosed by gavage with 28 mg/kg of the ring-labelled isomers. The sulfotransferase inhibitors decreased total hepatic macromolecular binding of 2,4-DNT by 33 percent and of 2,6-DNT by 69 percent. Both inhibitors also decreased the hepatic DNA binding of 2,6-DNT by >95 percent. DCNP decreased the binding of 2,4-DNT to hepatic DNA by >84 percent, while PCP decreased it by 33 percent. These results suggest that sulfation is an important step in the hepatic covalent binding of reactive metabolites of the DNTs.

Microfloral metabolism and toxicity of xenobiotics may be influenced by the pectin content of diets, due to the influence of pectin on microfloral β-glucuronidase activity. To test this theory, DeBethizy et al. (1983) fed male Fischer 344 rats a purified diet (controls), a purified diet plus 5 percent or 10 percent citrus pectin, or either of two cereal-based diets that varied in pectin content. After 28 days, rats were given 10 or 75 mg/kg ^{3}H-labelled 2,6-DNT per os. Total hepatic macromolecular covalent binding of the 2,6-radiolabel was found to be independent of diet at the low dose. However, at 75 mg/kg, covalent binding was increased 40 and 90 percent over that of the purified diet by feeding 5 percent and 10 percent pectin, respectively. Animals fed the cereal-based diets had 135 to 150 percent higher covalent binding than controls. Glucuronidase and nitroreductase activities were increased 2 to 3 times in the cecal contents of pectin-fed rats, corresponding to a 2 to 3-fold increase in the quantity of anaerobic microflora. The authors suggest that the pectin component of the diet could affect the tumorigenic potential of 2,6-DNT.

Woolen et al. (1985) reported trace levels of 2,4- and 2,6-DNT detectable in blood samples of workers at an explosives factory.

4.1.3 Metabolism

The metabolism of 2,4-DNT occurs in two ways, a reduction of the nitro groups and/or an oxidation of the methyl group. One or both of the nitro groups may be reduced to aminonitrotoluenes, while the methyl group is oxidized to a benzyl alcohol. These reductive and oxidative products may undergo subsequent conjugation to form glucuronides, sulfates, and other compounds (Lee et al. 1978). Various authors have attempted to identify whether there are sex differences in the metabolism of the dinitrotoluenes, as well as the products of DNT metabolism that are responsible for its apparent carcinogenicity. These include, in particular, diaminotoluene (DAT) and dinitrobenzaldehyde. Table 9 lists the principal metabolites of 2,4-DNT identified by various authors for in vivo and in vitro test systems. Although not listed as a category in the table, the largest proportions of metabolites were reported as unknowns in most of the studies.

4.1.3.1 In Vivo Studies

The major route of elimination of 2,4-DNT is via its metabolites in the urine (Lee et al. 1975; Ellis et al. 1978; Rickert and Long 1981; Mori et al. 1981; Schut et al. 1985). Similarly, 54 percent of the dose (10 mg/kg) of 2,6-DNT administered to male and female Fischer 344 rats was excreted as metabolites in the urine (Long and Rickert 1982).

Analysis of the urine from female rats treated with a single oral dose of ^{14}C-labelled 2,4- or 2,6-DNT indicated that extensive metabolism had occurred (Lee et al. 1975). Ellis et al. (1979) reported similar results after chronic feeding, with very little unmetabolized 2,4-DNT excreted in urine of male or female rats.

The principal metabolites of 2,4-DNT identified in urine of Fischer 344 rats are 4-(N-acetyl)amino-2-nitrobenzoic acid (4AcA2NBA), 2,4-dinitrobenzoic acid (2,4DNBA), 2-amino-4-nitrobenzoic acid (2A4NBA), and 2,4-dinitrobenzoic alcohol glucuronide (2,4DNBAlcG) (Rickert and Long 1981). Females excreted up to twice as much 2,4DNBAlcG as males (Rickert and Long 1981; Medinsky and Dent 1983). Schut et al. (1985) identified similar metabolites in urine of Strain A/J mice following intraperitoneal or oral dosing with ^{3}H-labelled 2,4-DNT, as well as small amounts of 2,4-diaminotoluene (2,4DAT) and the parent compound, 2,4-DNT. The amount of radioactive material present in the unconjugated fraction was less than ten percent of the dose for both intraperitoneal (i.p.) and oral administration, while that present in the glucuronide fraction was 2.4 to 7.5 percent of the i.p. dose and 20.5 to 28.2 percent of the oral dose.

Shoji et al. (1985) suggest that 2,4-dinitrobenzaldehyde (2,4DNBAld) might be an intermediate in the metabolism of 2,4-dinitrobenzyl alcohol (2,4DNBAlc) to 2,4-dinitrobenzoic acid, but were unable to identify it in the urine of male Wistar rats following oral administration of 2,4-DNT. However, in a subsequent study Shoji et al. (1987) demonstrated that 2,4 2,4DNBAld can be produced in vitro by

TABLE 9. METABOLITES OF 2,4-DINITROTOLUENE

Metabolite	Experimental System							
	Human	Rat	Mouse	Rabbit	Dog	Monkey	Hepatocytes	Microflora
2,4-Diaminotoluene		3,4	1,4	4	4	4		11,12
2,4-Diaminobenzyl alcohol		4	4	4	4	4		
2-Amino-4-nitrotoluene		2,3,4,6	1,4	4	4	4	1,7,9	1,11,12
4-Amino-2-nitrotoluene		2,3,4,13	1,4	4	4	4	1	1,11,12
2-Amino-4-nitrobenzoic acid	5,14	2,6	1					8,10
4-Amino-2-nitrobenzoic acid	5,14							
2-Amino-4-nitrobenzyl alcohol	3,4,6	1,4	4	4	4	7,9		
4-Amino-2-nitrobenzyl alcohol		3						
2,4-Dinitrobenzyl alcohol		3,4,13	1,4	4	4	4	1,7	1,10
2,4-Dinitrobenzyl alcohol glucuronide	5	2,6,13					7,9	8,10
2,4-Dinitrobenzoic acid	5,14	2,4,6,13	1,4	4	4	4	9	8
2-Acetylamino-4-nitrobenzoic acid	5							
4-Acetylamino-2-nitrobenzoic acid	5,14	2,6	1					8,10
2-Acetylamino 4-nitrotoluene		1,6						
4-Acetylamino-2-nitrotoluene							1,7	
2-Nitro-4-acetylaminotoluene		3						
2-Amino-4-acetylaminotoluene		3						
2-Amino-4-acetylaminobenzoic acid		3,13						
2-Nitro-4-nitrosotoluene								11
4-Nitro-2-nitrosotoluene								11

1. Schut et al. 1985.
2. Rickert and Long 1981.
3. Mori et al. 1981.
4. Lee et al. 1978.
5. Levine et al. 1985.
6. Medinsky and Dent 1983.
7. Bond and Rickert 1981.
8. Rickert et al. 1981.
9. Bond et al. 1981.
10. Rickert et al. 1983.
11. Guest et al. 1982.
12. Mori et al. 1985.
13. Shoji et al. 1985.
14. Woolen et al. 1985.

incubating 2,4DNBAl with microsomal and cytosol preparations. The interest in this compound stemmed from their earlier studies, which found that 2,4DNBAld had high mutagenic activity (Mori et al. 1982), and the possibility that the hepatocarcinogenicity of 2,4-DNT might involve the formation of 2,4DNBAld in the liver.

Long and Rickert (1982) also identified 2,6-dinitrobenzoic acid (2,6DNBA), 2,6-dinitrobenzyl alcohol glucuronide (2,6DNBAlcG) and 2-amino-6-nitrobenzoic acid (2A6NBA) in urine of male and female Fischer 344 rats following administration of radiolabelled 2,6-DNT. The metabolites detected accounted for 96 percent of the urinary radiolabel recovered, and there were no sex-dependent differences in the amounts of each metabolite excreted. Contrary to studies with 2,4-DNT, no acetylamino nitrobenzoic acid was detected in urine of rats administered the 2,6- isomer.

In a study using female Charles River CD rats, albino CD-1 and B6C3F1 mice, beagle dogs, New Zealand rabbits, and rhesus monkeys, Lee et al. (1978) reported similar metabolism of 2,4-DNT by all species studied. The 2,4DNBAlc and its glucuronides represented 23 to 44 percent of total radioactivity recovered in urine of the five species studied, with 2,4-aminonitrobenzyl alcohol (2,4ANBAlc) the second largest contributor to total activity recovered. The percent of administered dose recovered as 2,4-diaminotoluene was 0.6, 8.8, 4.2, 4.0, and 3.8 in mice, rats, rabbits, dogs, and monkeys, respectively.

The role of DNT pretreatmant and intestinal microflora in the reduction of 2,4-DNT was studied using conventional and axenic Fischer 344 rats (Rickert et al. 1981). No sex differences were seen in total percentage of dose excreted by all treatment groups, or in DNT metabolism in conventional rats with 30-day DNT pretreatment (35 mg/kg/day of a mixture of DNT isomers) followed by a single oral dose of 35 mg/kg 2,4-DNT. The percent of total dose excreted in urine of axenic rats of both sexes was about 2/3 that excreted in the urine of conventional rats. In addition, metabolites arising from 2,4-DNT oxidation (2,4DNBA and 2,4DNBAlcG) were excreted in similar quantities in the urine of conventional and axenic rats. However, metabolites requiring both oxidation and reduction [4-(N-acetyl)amino-2-nitrobenzoic acid and 2-amino-4-nitrobenzoic acid] were excreted in much smaller quantities in axenic rats. This indicates that intestinal microflora may play an important role in the reductive metabolism of 2,4-DNT.

The metabolism of radiolabelled 2,4- or 2,6-DNT in the small intestine of male Fischer 344 rats was studied by Rickert et al. (1983). Radioactivity moved rapidly through the intestine, with activity peaking in the last half of the small intestine and the cecal contents at 4 to 8 hr and 8 hr for 2,4- and 2,6-DNT, respectively. Both isomers at both doses studied behaved similarly. Four metabolites appeared in the intestine and its microfloral contents (Table 9), with peak concentrations of the glucuronides, 2,4- and 2,6DNBAlcG, occurring at 4 hr and between 4 and 8 hr, respectively. For each isomer, nearly all of the glucuronide disappeared within 12 hr of dosing. Covalent binding of 2,4-DNT radiolabel to macromolecules in cecal contents was detected 4 hr

after dosing, and concentrations were 2.5-fold higher than concentrations of 2,6-DNT radiolabel at 12 hr after dose. No appreciable evidence of 2,6-DNT covalent binding to cecal macromolecules appeared until 8 hr after dosing.

Metabolites identified in plasma following oral administration of 2,4-DNT to male or female Fischer 344 rats were 2,4DNBA and 2A4NBA, with no appreciable sex differences seen in concentration or elimination (Rickert and Long 1980). The sum of radioactivity recovered in these two metabolites represented only about 10 percent of the total radioactivity in plasma, but the authors were unable to identify the remaining compounds. Concentrations of 2,4-DNT and assayable metabolites were found to be measurably higher (2 to 10 times) in male than in female kidneys, with the sum of all assayable metabolites accounting for half the radioactivity in the male kidney but only 10 percent or less in the female kidney. Based on these results, the authors suggest that a significantly larger fraction of 2,4-DNT is metabolized to unknown metabolites in female rats than in male rats. No evidence of 2,4DAT (a suspected carcinogen) was found in any tissues (Rickert and Long 1980).

^{14}C-labelled 2,4-DNT was administered to pregnant Fischer 344 rats on gestation day 20 (Rickert et al. 1980). Metabolites detected in small amounts in maternal plasma were 2-amino-4-nitrotoluene, 2,4-dinitrobenzoic acid, and 2-amino-4-nitrobenzoic acid. Only 2,4-DNT was detected in placenta and amniotic fluid, accounting for 10 to 50 percent of the total radioactivity.

4.1.3.2 In Vitro Studies

The in vitro metabolism of 2,4-DNT has been studied extensively, and these studies confirm the results of in vivo metabolism studies. Table 9 lists the metabolites identified in rat liver homogenates (hepatocytes) and intestinal incubations (microflora).

In general, under anaerobic conditions, 2,4-DNT is predominantly reduced to 4-amino-2-nitrotoluene (4A2NT) and 2-amino-4-nitrotoluene (2A4NT) in liver homogenate (Lee et al. 1978; Kozuka et al. 1978; Mori et al. 1980, 1981; Decad et al. 1982), with liver tissue from males producing more aminonitrotoluenes than that from females (Lee et al. 1978). Little or no 2,4-dinitrobenzyl alcohol (2,4DNBAlc) was detected in anaerobic hepatocyte incubations (Lee et al. 1978; Decad et al. 1982). Using reductive reactivity indices, Kozuka et al. (1978) determined that the 4-NO_2 functional group of 2,4-DNT was more reactive than the 2-NO_2 group. Rats pretreated for 4 months with a diet containing 0.5 percent 2,4-DNT yielded significantly less 2A4NT and 4A2NT than untreated controls in anaerobic incubations of liver homogenate following a single oral dose of 50 mg/kg ^{3}H-labelled 2,4-DNT. This indicates the possibility of a saturation level for reduction of DNT in the pretreated liver (Mori et al. 1980).

In aerobic incubation of liver homogenate, oxidation is the principal reaction, with 2,4DNBAlc the major metabolite produced (Lee et al.

1978; Bond and Rickert 1981; Decad et al. 1982; Schut et al. 1985). Bond et al. (1981) reported oxidation, conjugation, and acetylation of 2,4-DNT in isolated rat liver, again with a low capacity for reduction. The major route of metabolism was oxidation of 2,4-DNT to 2,4DNBAlc and glucuronidation to 2,4DNBAlcG in both sexes. Following infusion of 20 μM 2,4-DNT, peak concentrations of DNBAlc were about 7 times higher in female than in male perfusate, while male livers excreted about 2.5 times more 2,4DNBAlcG in the bile than female livers. Female liver perfusate contained over 3 times as much of the glucuronide as male livers at the same 2,4-DNT concentration. After perfusion with 70 μM 2,4-DNT, the amounts of 2,4DNBAlcG in the bile and perfusate were similar for both sexes, indicating that a saturation level for the active transport of DNBAlcG into bile in males had been reached.

In order to determine the reductive metabolism of 2,4-DNT, oxygen concentrations in hepatocyte incubations were decreased (Bond and Rickert 1981); the presence of oxidation products (2,4DNBAlc) decreased, and the presence of reduction products (2A4NT and 4A2NT) increased. However, the small quantities of reduction products indicate that hepatic reduction of 2,4-DNT may not be an important metabolic pathway.

Prolonged anaerobic incubation of isolated rat hepatocytes did not result in the formation of 2,4-diaminotoluene (2,4DAT) from such precursors as 2A4NT or 4A2NT (Bond and Rickert 1981; Mori et al. 1984). The microsomal metabolism of 2,4-DNT was blocked by carbon monoxide or primary amines, indicating that cytochrome P-450 mediated the response. On the other hand, the cytosol fraction was able to metabolize 2,4-DNT to 2,4DAT via the aminonitrotoluenes, probably due to the activity of cytosolic xanthine oxidase since the metabolism was blocked by allopurinol (Mori et al. 1981). Treatment of rats with Aroclor 1254, phenobarbital, or 3-methylcholanthrene enhanced formation of 2,4DNBAlc in hepatocytes, while in vitro addition of SKF 525-A or 7,8-benzoflavone inhibited its formation, suggesting that the methyl-group hydroxylation of 2,4-DNT is accomplished by both a cytochrome P-450 dependent and a P-448 dependent mono-oxygenase (Bond and Rickert 1981).

The role of cecal microflora in the metabolism of 2,4-DNT was studied for male and female Fischer 344 rats, male Swiss-Webster mice, and human ileal and cecal contents (Guest et al. 1982). No sex- or species-specific differences in metabolism of 2,4-DNT were observed, and only slight species differences were seen in rate of metabolism, with mouse cecal contents the most active and human cecal contents the least. In addition, no metabolism of DNT was seen in aerobic incubations of cecal microflora for up to 3 hr. Anaerobic incubations of cecal microflora from male rats resulted in complete consumption of 2,4-DNT by 20 min. The first metabolites appearing were 2-nitro-4-nitrosotoluene and 4-nitro-2-nitrosotoluene, declining over time, with a corresponding increase in the amount of 2A4NT and 4A2NT seen. These aminonitro compounds subsequently declined, and 2,4DAT was detected. The authors were unable to isolate the intermediate hydroxylamino-amino compounds, and they postulated that if these compounds are sufficiently stable, they may covalently bind to microflora or hepatic macromolecules. Mori et al. (1985) also reported a sequential reduction of 2,4-DNT to 2A4NT and

4A2NT in cecal microflora of male Wistar rats, and were able to identify the intermediates, 4-hydroxylamino-2-nitrotoluene (2HA4NT) and 4-hydroxylamino-2-nitrotoluene (4HA2NT), but not the nitrosonitrotoluenes. The two aminonitrotoluenes were further reduced to 2,4DAT, and the authors suggested that the formation of 2,4DAT may be involved in the carcinogenicity of 2,4-DNT.

Similarly, Schut et al. (1985) investigated the ability of intestinal explants or cecal contents to metabolize 2,4-DNT (see Table 9). As with liver homogenate, under aerobic conditions, the most abundant metabolite formed by the small intestine was 2,4DNBAlc.

Aerobic incubations of Escherichia coli from human intestine with 2,4- and 2,6-DNT resulted in the formation of hydroxylaminonitrotoluenes and monoaminonitrotoluenes (Mori et al. 1984). The authors identified 4HA2NT, 2HA4NT, 2A4NT, and 4A2NT from incubations of E. coli with 2,4-DNT, and 2HA6NT and 2A6NT from incubations with 2,6-DNT.

4.1.3.3 Human Studies

Seventeen workers at a DNT manufacturing plant provided urine samples regularly over a 72-hr period (three work shifts) in an attempt to assess exposure to DNT (Levine et al. 1985; Turner et al. 1985). Three operators and a loader excreted 89 percent of the total metabolites (nmoles) measured from all workers. Peak excretion of metabolites in these four workers occurred during end-of-shift collection periods. There was no substantial evidence of accumulation of metabolites from day to day, and no apparent difference in the time course of elimination of 2,4- or 2,6- metabolites in these four workers. 2,4-DNT metabolites measured in urine of all workers are listed in Table 9; also included were 2,6-dinitrobenzoic acid (DNBA) and 2,6-dinitrobenzyl alcohol glucuronide (DNBAlcG), although 2-amino-6-nitrobenzoic acid (a nitro group reduction metabolite of 2,6-DNT) was not detected. The presence of 2-amino-4-nitrobenzoic acid (2A4NBA) as a major metabolite in human urine indicates that nitro group reduction is occurring in humans. In contrast to most of the rat studies described above, some unmetabolized 2,4- and 2,6-DNT were identified in some of the samples, but reliable quantitation was impossible. Although the metabolites identified in human urine were qualitatively similar to those identified in rat urine, there were quantitative differences in the metabolites measured. Human males and females excreted almost equal fractions of 2A4NBA (37.2 and 37.6 percent, respectively) and 2-acetylamino-4-nitrobenzoic acid (0.8 and 0.3 percent, respectively). However, in male humans, over half (52.5 percent) of total metabolites excreted were 2,4- and 2,6DNBA, with only 9.5 percent 2,4- or 2,6DNBAlcG. In female humans, about one-third of the metabolites excreted were DNBA and one-third DNBAlcG (28.8 and 33.3 percent, respectively). In male rats, about 50 percent of the urinary metabolites were identified as DNBA, similar to human males; however, 2A4NBA represented only about 5 percent of the dose in rat urine, compared with almost 40 percent in humans of both sexes (Rickert and Long 1981). As in rats, female humans excreted much more DNBAlcG in urine than male humans. Among all workers, and within individuals, there was considerable difference in the proportions of metabolites

excreted on any one day, often attributed to differences in exposure, but possibly due to individual differences in absorption, metabolic transformation, and excretion.

Two studies were conducted on 33 male and female workers exposed to tDNT at an explosives factory (Woolen et al. 1985). Routine air sampling indicated that atmospheric levels of DNT in the plant were well below the threshold limit value (TLV) of 1.5 mg/m^3 (0.03 to 0.1 mg/m^3). Air samples taken close to particularly dusty areas ranged from 0.02 to 2.68 mg/m^3 DNT. As in the above study, 2,4DNBA was the major metabolite identified in the urine, with a weekly mean post-shift concentration of 17 mg/L. No sex differences in 2,4DNBA excretion levels were noticed. There were, however, large intra- and inter-individual differences seen in concentrations measured in the urine. Other 2,4-DNT metabolites identified in the urine are listed in Table 9. Also identified was 2-amino-6-nitrobenzoic acid. No 2,4- or 2,6-dinitrobenzyl alcohol and only trace amounts of 2,4- or 2,6-DNT could be detected in the urine. The authors suggest that inhalation alone could not account for the levels of DNT and metabolites measured in the urine, and that another major route of DNT exposure, probably skin absorption, occurs.

4.1.4 Excretion

The principal route of excretion of 2,4-DNT and its metabolites in male and female Fischer 344 rats is in the urine, with excretion virtually complete after 24 hr (Rickert and Long 1981; Medinsky and Dent 1983). Lee et al. (1975) found that the feces and GI tract of female Charles River CD rats dosed with radiolabelled 2,4-DNT contained 60 percent, and urine contained 29 percent, of recovered radiolabel after 4 hr. However, 24 hr after dosing, feces and GI tract contained 12 percent and urine 76 percent of recovered radioactivity. Results using radiolabelled 2,6-DNT were similar, with 84 and 38 percent of label in feces, and 10 and 60 percent in urine after 4 and 24 hr, respectively (Lee et al. 1975). In these studies, only a negligible amount of radioactivity from either the 2,4- or the 2,6- isomer was found in expired air.

The same results were obtained using female New Zealand rabbits, beagle dogs, and rhesus monkeys (Lee et al. 1978). Urinary excretion was 75 to 81 percent, fecal excretion was 3 to 9 percent, and GI tract contents 5 to 11 percent of total label 24 hr following oral administration of radiolabelled 2,4-DNT. Schut et al. (1982) reported similar results with Strain A/J mice, with more than 50 percent of the dose appearing in the urine after 4 hr following i.p. administration; contents of the large intestine were 2 to 10 percent of the injected dose. Appearance of radiolabel in urine was slower after oral dosing, with 29 and 66 percent of radiolabel measured after 4 and 8 hr, respectively. Fecal elimination of dose was minimal regardless of the route of administration (1.1 to 2.1 percent of dose).

Ellis et al. (1979) also recovered 68 to 88 percent of radioactivity in the urine of Charles River CD rats 24 hr after single oral administration of radiolabelled 2,4-DNT following a 3-, 9-, or 20-month

regimen of 2,4-DNT dosing. Some radioactivity was found in the GI tract and feces, and very small amounts of radioactivity were recovered in the tissues.

In contrast to the above studies, the primary route of excretion 24 hr after administration of radiolabelled 2,4-DNT has been reported as the feces in female Charles River CD rats and Charles River CD-1 and B6C3F1 strain mice (Lee et al. 1975), as well as in male Wistar rats (Mori et al. 1977). It is possible that the differences in reported primary route of excretion between species are due to differences between strains in absorption of DNT.

In a later study (Mori et al. 1980) in which control male Wistar rats were held on normal diets for 4 months and then dosed orally with ^{3}H-labelled 2,4-DNT, similar results were reported, with about 34 percent of the administered dose excreted in feces and 20 percent excreted in urine after 7 days. The test rats for this study were dosed with 0.5 percent 2,4-DNT daily in feed for 4 months and then dosed orally with radiolabelled 2,4-DNT. Total excretion of radiolabel in urine and feces of test animals was slightly greater than for the control rats (about 59 and 54 percent, respectively), with more radiolabel excreted in urine than in feces.

Because 2,4-DNT has been found to be concentrated in the liver of various test animals, several investigators have studied the biliary excretion of ^{14}C-labelled 2,4-DNT and ^{14}C-labelled 2,6-DNT. Bile-duct-cannulated rats from which bile was collected excreted 20 to 60 percent of radioactivity in 36 hr, while recovery of the dose in cannulated rats in which bile was allowed to enter the intestines (controls) was 60 to 90 percent. In addition, male rats were found to excrete more radiolabel (25 percent) in bile than female rats (18 percent) following administration of radiolabelled 2,4-DNT, while female rats excreted more radiolabel in urine than male rats (Medinsky and Dent 1983). Excretion of radiolabel in bile was essentially complete after 24 hr in male rats and 12 hr in female rats.

Total recovery of radiolabel from 2,6-DNT in liver perfusate and bile was similar for male and female rats following incubation at 20 μM, but it was significantly greater in males than females after incubation at 70 μM (Long and Rickert 1982). This indicates the possibility of a saturation point in the metabolism and biliary excretion of 2,6-DNT in female rats. Female rats had more radiolabel in perfusate and significantly less ($p < 0.05$) radiolabel in bile than male rats at both concentrations tested. The major metabolite present in the bile of both sexes was 2,6DNBAlcG.

A dose of 100 mg/kg ^{14}C-labelled 2,4-DNT resulted in no sex differences in Fischer 344 rats in the excretion of 2,4DNBAlcG in urine, possibly indicating a saturation point in the formation or elimination of this metabolite in females (Rickert and Long 1981). Male Fischer 344 rats were found to excrete more radiolabel in bile (25 percent) than female rats (18 percent), with excretion complete after 24 hr in males and after 12 hr in females.

Slight differences in the excretion of orally administered 2,4- and 2,6-DNT in female Charles River CD rats have been reported by Lee et al. (1978). The rate of biliary excretion reached a peak in 1 to 4 hr following dosing for the 2,4- isomer, and in 6 hr for the 2,6- isomer. The blood concentrations of the radiolabel followed the concentrations in the bile for both isomers. Total radioactivity excreted in the bile after 24 hr was 10.9 and 24.8 percent of administered dose for 2,4- and 2,6-DNT, respectively. Radioactivity measured in the GI tract at this time was 7.6 and 6.9 percent of dose, respectively.

Urine was collected from employees at a DNT manufacturing plant and analyzed for DNT metabolites (Turner et al. 1985). Four determinations of total elimination half-times for the five metabolites identified in urine ranged from 0.88 to 2.76 days, indicating that DNT is rapidly excreted in the urine of humans. The half-time for elimination of 2,4DNBA from urine is given as 0.8 to 2.14 hr. Woolen et al. (1985) list the elimination half-time for 2,4DNBA as 2 to 5 hr in humans, and they suggest a biphasic elimination of the metabolite from urine.

4.2 ACUTE AND SUBACUTE TOXICITY

Unless otherwise indicated, the 2,4-DNT used in the studies described below contained 1 to 2 percent 2,6-DNT. Technical grade DNT contains about 19 percent 2,6-DNT. The following discussion will illustrate the carcinogenic significance of the 2,6- isomer (see Sect. 4.6).

4.2.1 Human Studies

No human epidemiological or laboratory studies are available that document the acute effects of DNT toxicity.

4.2.2 Animal Studies

Lethality data for 2,4-DNT are listed in Table 10. Oral LD_{50} values for 2,4-DNT range from 268 to 650 mg/kg for rats, and from 1250 to 1954 mg/kg for mice, indicating that rats are more susceptible to 2,4-DNT toxicity than mice. Toxic signs are central nervous system depression resulting in ataxia, respiratory depression, and death after a few hours (Ellis et al. 1980). Lee et al. (1975) reported that death in rats and mice occurred usually within the first 24 hr following acute administration of 2,4-DNT, with surviving animals completely recovered within 48 hr. No treatment-related gross pathology was observed in animals that had died.

LD_{50} data for 2,6-DNT are also listed in Table 10. Male rats seem to be slightly less tolerant to 2,6-DNT than 2,4-DNT, with an LD_{50} value of 535 mg/kg for 2,6-DNT, while female rats are more tolerant to this isomer (with an LD_{50} of 795 mg/kg). Both sexes of mice tested were less tolerant of the 2,6- than the 2,4- isomer, with LD_{50} values ranging from

TABLE 10. 2,4- AND 2,6-DINITROTOLUENE LETHALITY DATA

Species	Route/Vehicle	LD_{50} (mg/kg)		Reference
		2,4-DNT	2,6-DNT	
Charles River CD rat	Oral/peanut oil	650 (female)	795	Lee et al. 1975
	Oral/peanut oil	568 (male)	535	Lee et al. 1975
Sprague-Dawley rat	Oral	270	180	Vernot et al. 1977
Rat	Oral	268	-	ITII 1975
	Oral	-	177	Tatken and Lewis 1983
	-	1920	-	Vasilenko and Kovalenk
CF-1 mouse	Oral	1630 (male)	1000	Vernot et al. 1977
Swiss albino mouse	Oral/peanut oil	1340 (female)	807	Lee et al. 1975
	Oral/peanut oil	1954 (male)	621	Lee et al. 1975
Mouse	Oral	1625		ITII 1975
	Oral	1250		Tatken and Lewis 1983
	-	790		Vasilenko and Kovalenk
Guinea pig	Oral	1300		Tatken and Lewis 1983
Rabbit	-	620		Vasilenko and Kovalenk
Mammal	Subcutaneous	50		Tatken and Lewis 1983

621 to 1000 mg/kg. Time to death, recovery, and gross pathology were the same for 2,6-DNT as for 2,4-DNT (Lee et al. 1975).

Spector (1956) reported lethal doses for cats of 50-500 mg/kg (subcutaneous) and 60 mg/kg (intraperitoneal) for 2,4- and 2,6-DNT, respectively.

As early as 1919, the toxic effects of DNT on the vascular system, blood pressure, and survival in laboratory animals were studied (Perkins 1919). The "oil" of dinitrotoluene was injected subcutaneously in dogs and found to result in death after 20 hr following a single dose of 0.5 mg/kg. No effects were seen at 0.2 mg/kg. Lesions in the liver and slight alterations in the kidneys were seen in fatal cases. Congestion, cytological injury, alterations in the chondriosome, and abnormal inclusions were reported for the liver. In a further test, the 2,4- and 2,6- isomers were injected subcutaneously in dogs. 2,4-DNT was found to result in death after 8 hr at a single dose of 0.5 mg/kg and death in 4 days at a dose of 0.2 mg/kg, with no effect at 0.1 mg/kg. For 2,6-DNT, survival in dogs was reported for an injected dose of 0.05 mg/kg, and death after 2 or 8 days for an injected dose of 0.1 mg/L, indicating that the 2,6- isomer is slightly more toxic in dogs. Doses of about 0.003 g/kg of the "oils of toluene" injected under the skin or into the peritoneum of rabbits and exposure to vapors were found to have no vasodilatory effects.

Vasilenko (1978) reported that following an oral dose of 50 percent of the LD_{50} (dose unspecified), 2,4-DNT induced 15 percent methemoglobinemia and >4 percent sulfhemoglobinemia in rats. Other indications of hemotoxicity were decreases in blood SH group content, presence of Heinz bodies, and reticulosis with increased incidence of acid resistance of erythrocytes. Vasilenko also reported alterations in bound transferrin and ceruloplasmin.

McGown et al. (1983) studied the effects of 2,4-DNT on tissue histology, blood chemistry, hematology, and urinary parameters. Male and female Sprague-Dawley rats were fed 2,4-DNT at the rate of 0, 0.9, 1.2, 1.9, or 3.0 g/kg diet for 14 days (Table 11). Assuming an average total dietary intake of 0.0175 kg/day, and an average weight of 0.35 kg (USEPA 1986), this represents a daily intake of 45, 60, 94, or 143 mg/kg/day, respectively. No DNT-related effects were seen on blood hematology or urinary parameters; however, dose-related increases in blood glucose in both sexes, increases in blood cholesterol in females, and elevated alanine aminotransferase in males were reported. Hyaline droplet formation (not dose-dependent) in the epithelium of the proximal convoluted tubules of the kidneys was seen in both sexes, with males more susceptible than females.

A photocontact allergy to dinitrotoluene in a man who used dynamite in his work as a rockblaster was reported by Emtestam and Forsbeck (1985). When the worker began wearing gloves during his blasting activities, his dermatitis disappeared.

TABLE 11. SUMMARY OF 2,4-DNT[a] SUBCHRONIC TOXICITY STUDIES

Species	Daily Dose (mg/kg/day)	Duration (weeks)	Effects
Dog			
Beagle[b,c]	25	13	Lethal after 22 days for 50% males and females; neuromuscular effects; CNS lesions; methemoglobinemia with Heinz bodies; anemia; reticulocytosis, hemosiderosis, extramedullary hematopoiesis; decreased spermatogenesis
	5	13	NOEL[d]
Rat			
Charles River CD[b,c]	266[e] or 145[f]	13	All females died by week 3; 8/16 males died by week 13; weight loss; greatly reduced food consumption; severe testicular atrophy and aspermatogenesis; hemosiderosis
	93[e] or 108[f]	13	Decreased weight gain; reduced food consumption, anemia with compensatory reticulocytosis in male rats; hemosiderosis; moderate to severe testicular atrophy and aspermatogenesis.
	34[e] or 38[f]	13	Slightly decreased weight gain; reduced food consumption; slight reticulocytosis
Wistar STD8	190-214	26	12/20 Deaths; piloerection, whitened skin color, humpback, jerky movement, general weakness; methemoglobinemia; increased relative liver, spleen, and kidney weights; decreased body and testicular weight; puroploid matters in liver, testicular atrophy; increased activity of triglyceride, blood glucose, and serum enzymes (except SGPT[h]) levels
Sprague-Dawley[i]	116[e] or 147[f]	2	Elevated alanine aminotransferase in males; elevated glucose levels and albumin/globulin ratio in females; increased cholesterol in both sexes; dose-dependent decrease in weight gain correlated with a dose-related depression in food consumption; dose-related oligospermia, syncytial cell formation, degenerative changes in testes
	116[e] or 147[f]	2	Elevated alanine aminotransferase in males; elevated albumin/globulin ratio in females; increased cholesterol in both sexes
	73[e] or 93[f]	2	Elevated alanine aminotransferase in males; increased cholesterol in both sexes
Mouse			
Swiss albino[b,c]	35 or 46,[e] 44 or 60[f]	13	Weight loss; little or no body fat, anemia with compensatory reticulocytosis; mild depression of spermatogenesis
	137[e] or 147[f]	13	NOEL

a. 2,4-DNT = 2,4-dinitrotoluene.
b. Dogs were dosed daily by capsule; rats and mice were dosed daily with 2,4-DNT concentrates in feed.
c From Lee et al. 1978.
d. NOEL = no observed effects level.
e. Dose to males.
f. Dose to females.
g. From Kozuka et al. 1979.
h. SGPT = serum glutamic pyruvic transaminase.
i. From McGown et al. 1983.

Skin irritation tests in rabbits showed 2,4-DNT to be a very mild primary skin irritant, although it was less irritating than the peanut oil control. Eye irritation tests in rabbits and maximum sensitization tests in guinea pigs were also negative for 2,4-DNT. 2,6-DNT was a borderline mild skin irritant in rabbits, and a mild sensitizing agent in guinea pigs, but was nonirritating to the rabbit eye (Lee et al. 1975).

In an effort to determine the ability of DNT isomers to alter the metabolism of xenobiotics, oral doses of 2,4-DNT were administered to male Charles River CD rats twice daily for 3 days, and once on the fourth day. 2,4-DNT had no pronounced effect on zoxazolamine paralysis time or hexobarbital sleeping time (Short and Lee 1980). Lee et al. (1978) obtained the same results feeding 2,4-DNT in the diet to male Charles River CD rats for two weeks. Zoxazolamine paralysis time was unaffected in treated rats, and in vitro measurements of hepatic nitroanisole O-demethylase activity were similar between treated animals and controls. However, treatment with 2,6-DNT was found to induce a biphasic change in the liver metabolism of xenobiotics in rats, significantly increasing the times when compared with controls following 3-day treatment. Male rats fed 2,6-DNT daily for 4 weeks showed significantly reduced zoxazolamine paralysis time and nitroanisole O-demethylase activity of the postmitochondrial supernatant when compared with controls. Statistical analyses of results were performed using a two-sample rank test (Short and Lee 1980).

4.3 SUBCHRONIC AND CHRONIC TOXICITY

4.3.1 Human Studies

A study of munitions-related intoxication in France during the First World War was reported by Perkins (1919). During the manufacturing process for dinitrotoluene, oily byproducts were removed in an attempt to reduce its toxicity. During a 6-month period in 1915, about 100 tons of "drained" dinitrotoluene were produced at a French factory. The drainage process consisted of washing off the oily isomeric byproducts, pouring the crude, washed DNT into buckets of iron, and allowing the DNT to solidify. The solidified cakes of DNT were then broken up and left on a drying rack for 24 hr. Several cases of intoxication were seen during the washing and draining process, particularly during the physical breaking of the cakes. It was reported that on several occasions 50 percent of the 25-man workforce was incapacitated. Symptoms recorded were cyanosis, dizziness, and a tendency to sleep, with headache, dyspnea, and brown urine. Recovery occurred 2 to 3 days after exposure, and more quickly if the subject left the area immediately. Alcoholic subjects were quite sensitive to toxicity. Aching in joints, particularly the knees, was reported, continuing in some cases for several months after exposure. No fatal accidents were recorded. Exposure was thought to occur through the skin and the respiratory and digestive tracts.

The toxicity of DNT to humans was further documented in 154 case reports from workers engaged in the application of DNT to the manufacture of military explosives during the early 1940s (McGee et al. 1942). Each of the employees worked in either a DNT screening house or a coating house, and none had been previously exposed to DNT. Of the 154 men studied, 42 at no time reported feeling unwell, although 4 of those developed an anemia. Eighty more of the workers had objective signs of sickness, while 32 reported complaints but had no clinical evidence of intoxication or illness. An unpleasant taste in the mouth was the most frequent symptom, occurring in 62 percent of the cases. This was followed in frequency by muscular weakness (51 percent), loss of appetite (49 percent), dizziness (47 percent), nausea (44 percent), insomnia (37 percent), and pain in the extremities, occasionally localized in joints (26 percent). Other symptoms occurring in less than 25 percent of the cases included vomiting, numbness and tingling, loss of weight, and diarrhea. Clinical examination found the chief effects to be pallor (36 percent), cyanosis (34 percent), and anemia (23 percent). Leucocytosis, hypotension, skin rash, leucopenia, and acute toxic hepatitis (2 individuals) occurred in 12 percent or less of the workers. The beginning symptoms of toxicity usually disappeared within 2 to 3 days after removal from exposure; however, McGee et al. (1942) found that the blood effects often took more than 2 months post exposure to return to normal. Six instances of dermatitis attributable to DNT exposure were reported. In the two most severe cases, acute damage to the hematopoietic system and reversible acute liver and kidney damage were evident. As reported earlier, alcohol aggravated the symptoms recorded, with reactions including substernal pressure, precordial palpitation, fullness in the head, and severe, acute illness. The authors noted a remarkable variation in the susceptibility of workers to DNT intoxication. Following the implementation of proper ventilation, personal hygiene methods including adequate washing of hands, and the application of useful engineering and operating principles, the incidence of DNT poisoning was reduced (McGee et al. 1947). Anemia was reported in the later study in 10.2 percent of the 714 workers observed, cyanosis in 8.7 percent, and dermatitis in 4.5 percent.

Recently, Levine et al. (1986) examined mortality data from cohorts of 156 and 301 men working a month or more in the 1940s and 1950s at jobs related to the production and use of DNT. At one plant, workers were potentially exposed to tDNT containing 19 percent 2,6-DNT as well as 2,4-DNT containing 1 percent 2,6-DNT, while at the other plant workers were only exposed to the latter. Workers at the first plant also had the opportunity for exposure to TNT. The median time for employment at the plants was 2.1 yr and 1.8 yr, respectively, while the median time for employment at jobs with the potential for exposure to DNT was 0.4 yr and 1.2 yr, respectively. There was an increased mortality rate for all the men studied, compared with the standard for white males in the United States. This was due largely to increased mortality from circulatory diseases, in particular ischemic heart disease. Although mortality from ischemic heart disease is elevated in persons living in areas surrounding the two plants, mortality from ischemic heart disease observed in workers was still high compared with the local mortality. At both plants mortality from ischemic heart disease was less than expected

during the first 15 yr following cohort entry, increasing only in later years. A relationship between mortality and duration and intensity of exposure was suggested by the data.

4.3.2 Animal Studies

Results of subchronic toxicity testing for both the 2,4- and the 2,6- isomer are summarized in Tables 11 and 12 and are discussed below.

4.3.2.1 Subchronic Studies

Lee et al. (1978) studied the subchronic effects of 2,4-DNT on dogs, rats, and mice using 4- and 13-week dosing schedules. Results of the 13-week tests are summarized in Table 11. Beagle dogs fed 1 or 5 mg/kg/day were healthy throughout the treatment period, while the high-dose (25 mg/kg/day) dogs were in fair to poor nutritional state, with little body fat, and showed neuromuscular incoordination and paralysis. Daily treatment of 25 mg/kg/day resulted in toxic signs from 12 to 22 days and was lethal to 50 percent of the dogs after 22 days. The three primary targets were the neuromuscular system, the erythrocytes, and the testes. Partial recovery of dogs was seen 4 weeks after treatment was discontinued. The immunologic response to 2,4-DNT was determined in dogs by measuring the quantity of immunoglobin E (IgE) in the blood after 4 and 13 weeks of exposure, and after 4-week recovery following exposure. There were no apparent changes in serum concentration of IgE during any of the exposure and recovery periods, indicating no anaphylactic reaction to DNT.

Rats fed the low dose of 34.3 (males) or 38.3 (females) mg/kg/day exhibited minimal adverse effects, including a slight decrease in weight gain, while those fed the higher levels of 2,4-DNT exhibited a greater decrease in weight gain, reticulocytosis, hemosiderosis in the spleen, and decreased spermatogenesis (Table 11). All of the females treated at the high dose level (145 mg/kg/day) died within 3 weeks, and half of the high-dose (266 mg/kg/day) males died before the end of the experiment. A few intermediate- and high-dose animals exhibited neuromuscular effects similar to those seen in dogs, with the exception of the paralysis, and some had mild to moderate gliosis and/or demyelinization (Lee et al. 1978). Partial recovery was seen in rats 4 weeks after treatment was discontinued.

The studies with mice showed them to be more tolerant of 2,4-DNT toxicity than rats or dogs (Lee et al. 1978). The low and middle dose levels (47 to 147 mg/kg/day) proved to be nontoxic, while the high dose level (413 to 468 mg/kg/day) caused weight loss, mild anemia, and only a few deaths. A mild depression of spermatogenesis was seen in two mice after 4 weeks of exposure, but testicular lesions were not seen in any male mice after 13 weeks. Most mice fed the high dose had little or no body fat after 4 or 13 weeks. Complete recovery in mice was seen in 4 weeks following discontinuation of treatment.

TABLE 12. SUMMARY OF 13-WEEK 2,6-DNT[a] TOXICITY STUDIES[b]

Species	Daily Dose[c] (mg/kg/day)	Effects
Dog		
Beagle		
	100	Listless; incoordination with rigid paralysis; pale gums; 100% mortality by week 8; extreme weight loss with little body fat; severe anemia; leucocytosis; increased SGPT[d] until 4 wks; Heinz bodies; enlarged spleen; reduced testes weight; extramedullary hematopoiesis in liver and spleen; bile duct hyperplasia; degeneration and/or inflammation of liver; spermatogenesis reduced; involution of thymus; reduced lymph in spleen and lymph nodes
	20	Slight weight loss; 25% mortality in week 9; anemia with compensatory reticulocytosis; slight methemoglobinemia with Heinz bodies; increased SGPT; extramedullary hematopoiesis in liver and spleen; bile duct hyperplasia; degeneration and/or inflammation of liver; spermatogenesis reduced
	4	Mild splenic hematopoiesis; lymphoid depletion in spleen
Rat		
Charles River CD		
	144.7[e] or 155.0[f]	Weight loss; decreased erythrocyte count; compensatory reticulocytosis; methemoglobinemia and Heinz bodies; testicular, bile duct, and splenic lesions
	35.1[e] or 37.1[f]	Decreased weight gain and feed intake; elevated SGPT; extramedullary hematopoietic activity in spleen and/or liver; bile duct hyperplasia; depressed spermatogenesis; testicular atrophy
	7.2[e] or 7.4[f]	NOEL[g]
Mouse		
Swiss albino		
	288.8[e] or 298.8[f]	All males died before week 9; 6 out of 8 females died; low body weight; decreased feed consumption; same effects as for mid-dose level, but more severe
	50[e] or 55.2[f]	All males and 1 out of 8 females died; testicular atrophy; depressed spermatogenesis; bile duct hyperplasia; extramedullary hematopoiesis in liver and/or spleen
	11.0[e] or 11.1[f]	NOEL

a. 2,6-DNT = 2,6-dinitrotoluene
b. Adapted from Lee et al. 1976.
c. Dogs were dosed daily by capsule; rats and mice were dosed daily with 2,4-DNT concentrates in feed.
d. SGPT = serum glutamic pyruvic transaminase.
e. Dose to males.
f. Dose to females.
g. NOEL = no observed effects level.

Male Wistar-STD strain rats were fed a diet containing 0.5 percent 2,4-DNT for 26 weeks (Kozuka et al. 1979). Estimated ingestion was 66 mg/day for the first 3 months and 75 mg/day for the last 3 months. Assuming an average weight for rats of 0.35 kg (USEPA 1986), this represents a daily intake of 190 and 214 mg/kg/day, respectively. Effects seen in treated rats are listed in Table 11. Methemoglobin formation in treated animals was about 7 times that seen in controls. The dietary effects of 2,4-DNT on microsomal drug-metabolizing enzymes were also studied by Kozuka et al. (1979). They found no effects on aniline hydroxylase or aminopyrine N-demethylase activity, but they reported a marked decrease in p -nitrobenzoic acid reductase activity.

The subchronic effects of 2,6-DNT on dogs, rats, and mice were reported by Lee et al. (1976). Table 12 lists the results of their study. Higher doses were more toxic, with all dogs dosed with 100 mg/kg/day dying by week 8 of the study, and two out of eight dogs dosed with 20 mg/kg/day dying by week 9. Bile duct hyperplasia and lesions in the liver and spleen, as well as degenerative and inflammatory changes, were evident after treatment for 13 weeks at 20 or 100 mg/kg/day. Dogs treated at the two lower dose levels for 4 weeks or 13 weeks showed some recovery after 4 weeks with no additional exposure, and dogs treated with 100 mg/kg/day for 4 weeks showed complete recovery after 19 weeks.

Lee et al. (1976) also studied the effects of daily doses of 2,6-DNT on Charles River CD rats (Table 12). Contrary to the results seen in dogs, there were no overt signs of toxicity or unscheduled deaths; rats at the highest dose were less active. However, blood analysis and microscopic examination of tissue indicated that in rats the main effects of 2,6-DNT were quantitively and qualitatively similar to those of 2,4-DNT. Erythrocyte and testicular effects were virtually identical. Bile duct hyperplasia was seen in rats treated with 2,6-DNT.

Male and female albino Swiss mice were fed 2,6-DNT in rodent chow for 4 or 13 weeks (Table 12). The lowest dose level (11 mg/kg/day for males and females) produced no effects. The intermediate and high doses (50 and 289 mg/kg/day for males and 55 and 299 mg/kg/day for females) produced decreases in weight gain and feed consumption and increased deaths during the 13-week study. All males had died by the ninth week, and only two females survived until the end of the 13-week study. There were two unscheduled deaths in the control group, and three in the low-dose group at weeks 1 and 3 of the study, but the authors did not attribute these deaths to 2,6-DNT. There were no toxicologically significant changes seen in the peripheral blood elements, possibly due to collection difficulties. Mice fed the high dose level showed the same, but more severe, clinical effects as those fed the intermediate dose level.

4.3.2.2 Chronic Studies

In a 1-yr study designed to compare the carcinogenicity of 2,4-, 2,6-, and tDNT in male Fischer 344 rats, Leonard et al. (1987) noted significant reductions in body weight gain and increases in liver weight following administration of 27 mg/kg/day 2,4-DNT, 35 mg/kg/day tDNT, or

7 or 14 mg/kg/day 2,6-DNT. Serum alanine aminotransferase was elevated following administration for 52 weeks of both doses of 2,6-DNT, and serum gamma-glutamyl transferase was increased after 26 and 52 weeks at the high dose of 2,6-DNT. Neither parameter was affected by 2,4- or tDNT.

The effects of 2,4-DNT during 2-year feeding tests with dogs, rats, and mice were studied by Ellis et al. (1979), and are summarized in Table 13. Six male and six female beagle dogs were used in each dosage group studied. Treatment with 0.2, 1.5, or 10 mg/kg/day 2,4-DNT for 12 months did not have any obvious effects on organ weights, and had only minimal effects on tissues. One dog experienced bile duct hyperplasia and slight pigment deposits in the liver at the high dose. The results of this 12-month study are not included in Table 13. Doses of 10 mg/kg/day for 2 yr (Table 13) were found to be lethal to 50 percent of the dogs tested, causing neuromuscular effects and degenerative lesions in the cerebellum. Methemoglobinemia with the presence of Heinz bodies and reticulocytosis was seen, but these effects were minimal during the second year of study. A 1-month recovery period resulted in complete elimination of the anemia and sequelae. Histopathologic results are listed in Table 13. The bile duct and gall bladder lesions were relatively mild, but there was no indication of recovery following the 2-yr treatment. One of the six dogs dosed daily at 1.5 mg/kg exhibited a loss of muscular control and occasional convulsive tremors throughout the study. Mild hyperplasia of the bile duct and kidney epithelium was seen in a few animals at this treatment level. Dogs treated with 0.7 mg/kg/day for 2 yr exhibited no apparent effects.

Male and female Charles River CD rats were fed daily doses of 2,4-DNT for 2 yr (Ellis et al. 1979). Rats fed 0.57 (males) and 0.71 (females) mg/kg/day showed no apparent effects, while rats fed 3.9 and 5.1 mg/kg/day showed some mild effects in susceptible individuals. These effects are summarized in Table 13. Severely decreased weight gain, shortened life span, and various pathological effects were seen at 34 and 45 mg/kg/day for male and female rats, respectively. The three major causes of death were pituitary umors, ulcerated subcutaneous tumors, and loss of vigor. The effects of pituitary tumors on motor function included one-sided ataxia, paralysis, and exaggerated response to stimuli. The most significant tissue lesion was the progressive development of hepatocellular carcinoma (discussed in the section on oncogenicity). In contrast to the studies with dogs, although there was an anemia partially compensated by increased reticulocyte counts, there was no evidence of methemoglobinemia with the presence of Heinz bodies in the rats. All of the high-dose rats but one had died before the end of month 23, with only a few more deaths seen in the middle-dose group than in controls.

Ellis et al. (1979) also studied the chronic toxicity of 2,4-DNT in male and female Charles River CD-1 mice (see Table 13). The major targets were reported as the blood, the kidney, the liver, and the gonads, with male mice more affected than female mice. All of the male mice fed the high dose (900 mg/kg/day) died by month 18 of the study, and about 95 percent of the female mice fed the high dose died by month 21 of the

TABLE 13. SUMMARY OF 2,4-DNT[a] CHRONIC TOXICITY STUDIES[b]

Species	Daily Dose[c] (mg/kg/day)	Test Duration	Effects
Beagle dog	10	2 yr	Neuromuscular effects; CNS[d] lesions; cyanosis; methemoglobinemia with Heinz bodies and reticulocytosis; lethal to 50%; pigmentation in liver, kidney, gall bladder, and spleen; hyperplasia of bile duct and gall bladder epithelium
	1.5	2 yr	Some CNS effects; slight reduction in weight gain; LOAEL[e]
	0.2	2 yr	NOEL[f]
Charles River CD rat	34[g] or 45[h]	1 yr	Decreased body weight; increased liver and kidney weights, decreased testis weight; subcutaneous tumors; hyperplastic liver foci in 4/8 animals; neoplastic liver nodules in 8/8 animals; hepatocellular carcinoma, in 1/8; excessive pigmentation in spleen, in 7/8 animals; severe testicular atrophy, with almost complete lack of spermatogenesis, in 4/4
	34[g] or 45[h]	2 yr	Lethal to 75/76 rats; severely reduced weight gain; anemia; hyperplastic liver foci, neoplastic nodules, hepatocellular carcinoma; severe testicular atrophy in 86% of males; subcutaneous tumors in 75% of males, and 90% of females after 18 week
	3.9[g] or 5.1[h]	1 yr	Slightly lower weight after 9 months; mild degree of hyperplastic foci in liver of males
	3.9[g] or 5.1[h]	2 yr	Slightly more deaths than controls; subcutaneous tumors; occasional anemia; decreased body weight; increased liver weight with hyperplastic foci; testicular atrophy in 33% of males
	0.57[g] or 0.71[h]	1-2 yr	Mild degree of hyperplastic foci in liver of males; NOAEL[i]

TABLE 13 (CONTINUED)

Species	Daily Dose[c] (mg/kg/day)	Test Duration	Effects
Charles River CD-1 mice	900	1 yr	About 83% dead; decreased body and testes weights, increased liver weights; anemia with compensatory reticulocytosis; methemoglobinemia with Heinz bodies; testicular atrophy; non-functioning follicles lacking corpora lutea; pigmentation in many tissues; hepatocellular dysplasia and nephropathy
		2 yr	100 % mortality
	95	1 yr	Decreased weight gain; some methemoglobinemia with Heinz bodies
		2 yr	No significant differences in organ weights; decreased weight gain in males; kidney tumors in males; liver dysplasia in males; abnormal pigmentation in many tissues; testicular atrophy
	13.5	1 yr	NOEL
		2 yr	Lower body weight in males; liver dysplasia in males; kidney tumors in males; abnormal pigmentation in males and females

a. 2,4-DNT = 2,4-dinitrotoluene.
b. Adapted from Ellis et al. 1979.
c. Dogs were dosed daily by capsule; rats and mice were dosed daily with 2,4-DNT concentrates in feed.
d. CNS = central nervous system.
e. LOAEL = lowest observed adverse effects level.
f. NOEL = no observed effects level.
g. Dose to males.
h. Dose to females.
i. NOAEL = no observed adverse effects level.

study. After 1 yr, most of these mice exhibited anemia, with decreased erythrocytes and hemoglobin. A high level of Heinz bodies, increased reticulocytes, and occasional methemoglobinemia were seen. Similar effects were seen in some moribund mice dosed at 95 mg/kg/day. Kidney tumors appeared after 2 yr in surviving males. Mice fed 900 mg/kg/day experienced greatly decreased weight gain, increased liver weight, and decreased testis weight. A dose- and time-dependent increase in pigmentation, particularly of the spleen and liver, was reported for mice at all levels. The 1-month recovery studies after feeding 2,4-DNT for 12 or 24 months resulted in partial recovery from the toxic anemia, reduced severity of lesions, and statistically significant reductions in relative spleen weights (after 24 months exposure only).

The chronic toxicity of tDNT in albino Fischer 344 rats was studied by the Chemical Industry Institute of Toxicology (CIIT 1982). Table 14 summarizes the results. There were 130 rats of each sex in each dose group (0.0, 3.5, 14.0, or 35.0 mg/kg/day), some scheduled for interim sacrifice. All of the rats in the high-dose group were sacrificed after 55 weeks, because of histopathologic findings discovered earlier; at the end of the 104-week study, only 23 male rats (26 percent) survived in the mid-dose group, while survival of female rats in the mid-dose group and survival of males and females in the low-dose group were comparable to controls. Treatment-related effects of tDNT were hunched, thin, and/or bloated appearance, with a significant ($p = 0.05$) dose-related decrease in body weight gain in both sexes. There were gross alterations of the liver at all doses in both sexes, evident as a progressive hepatocellular carcinoma, and an increased incidence of benign subcutaneous tumors (discussed in Sect. 4.6). There was an increased incidence of testicular degeneration in mid- and high-dose males, with hypospermatogenesis apparent. Hematological effects were similar in all groups tested.

4.4 GENOTOXICITY

There have been many short-term assays utilized to determine the genotoxicity of the dinitrotoluene isomers. Table 15 lists the results of the Ames Salmonella assay with 2,4-DNT. Table 16 summarizes the results of other short-term tests for 2,4-DNT, which, for the most part, will not be discussed in the text.

4.4.1 Bacterial and Yeast Studies

Several investigators have used the Ames Salmonella assay to determine the mutagenic activity of 2,4-DNT (Table 15). In general, weak mutagenicity was reported in each of the base-pair substitution or frameshift strains tested with less, or no, activity in the presence of an S9 liver homogenate activation system.

The mutagenicity of 2,4- and 2,6-DNT using the Ames Salmonella test was reported by Spanggord et al. (1982), with positive results in TA100 indicating a reproducible dose-response curve over at least three dose

TABLE 14. SUMMARY OF tDNT[a] CHRONIC TOXICITY STUDIES IN FISCHER 344 RATS[b]

Daily Dose (mg/kg/day)	Test Duration	Effects
3.5	2 yr	White and/or yellow foci in liver; hepatotoxicity in males; increase in absolute liver weight; benign subcutaneous tumors in males
14.0	2 yr	Decreased red cell mass in males; increased mean reticulocyte values in males; increased SGPT[c] in males; white and/or yellow foci in liver; increased incidence of liver nodules or tissue masses; increase in absolute and relative liver weight; testicular atrophy; hypospermatogenesis; mammary fibroadenomas; subcutaneous fibromas; exacerbation of chronic interstitial nephritis
35.0	1 yr	Decreased red cell mass; increased mean leucocyte values; hepatotoxicity; increased mean reticulocyte values in males; increased SGPT[c]; white and/or yellow foci in liver; testicular degeneration, hypospermatogenesis; exacerbation of chronic interstitial nephritis in males; increase in absolute and relative liver weight; hepatic carcinoma in 20/20 males and 11/20 females.

a. tDNT = technical grade dinitrotoluene.
b. From CIIT 1982.
c. SGPT = serum glutamic pyruvic transaminase.

TABLE 15. MUTAGENICITY OF 2,4-DNT[a] IN THE *Salmonella typhimurium* REVERSION ASSAY

Reference	TA1535		TA100		TA1537		TA1538		TA98	
	+[b]	-[c]	+	-	+	-	+	-	+	-
Couch et al. 1981	Neg	2X	Neg	Neg	Neg	2X	Neg	3X	Neg	3X
Mori et al. 1982				<3X						<3X
Klopman et al. 1985			Neg						Neg	
Tokiwa et al. 1981			Neg	Pos					Neg	Neg
Spanggord et al. 1982	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg
Chiu et al. 1978				<2X						<2X
Doran et al. 1985				Pos				Pos		
Ellis et al. 1978	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Neg	Neg

a. 2,4-DNT = 2,4-Dinitrotoluene.
b. + = With metabolic activation.
c. - = Without metabolic activation.

TABLE 16. GENOTOXICITY OF 2,4-DINITROTOLUENE

Species/Cell System	Dose/ Concentration	Results	Reference
Micronucleus assay			
Mouse (CBA x BalbC)F_1	200 and 400 mg/kg	Neg.	Ashby et al. 1985
Unscheduled DNA synthesis			
Rat (Alderly Park)	100 and 200 mg/kg	Pos.	Ashby et al. 1985
(Charles River CD) spermatocytes	10 or 100 µM	Neg.	Working and Butterworth 1984
(Fischer 344) hepatocytes	10^{-4}M	Neg.	Bermudez et al. 1979
Human (primary hepatocytes)	1mM	Neg.	Butterworth et al. 1983
In vivo/in vitro DNA repair assay			
Rat (Fischer 344)	50 or 200 mg/kg	Pos.	Mirsalis, Tyson, and Butterworth 1982
	100 mg/kg	Pos.	Mirsalis and Butterworth 1982
Sperm abnormality assay			
Rat (Fischer 344)	25, 100, 150, and 200 mg/kg	Pos.	Ashby et al. 1985
P388 Mouse lymphoma mutation assay			
Mouse lymphoma cell line (TK +/-)	1.6-1000 µg/mL, without metabolic activation	Pos.	Styles and Cross 1983
Mouse lymphoma cell line (TK +/-)	1.6-1000 µg/mL, with metabolic activation	Neg.	Styles and Cross 1983
Sex-linked recessive lethal mutation			
Drosophila melanogaster	Not given; p.o.[a]	Neg.	Woodruff et al. 1985
	Not given; i.p.[b]	Pos.	Woodruff et al. 1985

TABLE 16 (CONTINUED)

Species/Cell System	Dose/ Concentration	Results	Reference
Recriprocal translocation test			
Drosophila melanogaster	Not given; i.p.[a]	Neg.	Woodruff et al. 1985
Sperm morphology test			
Mouse (DBA/2J)	250 mg/kg p.o.[b] or i.p.	Neg.	Soares and Lock 1980
Dominant lethal assay			
Mouse (DBA/2J)	250 mg/kg p.o. or i.p.	Neg.	Soares and Lock 1980
	250 mg tDNT/kg p.o. or i.p.	Neg.	Soares and Lock 1980
Rat (Sprague-Dawley)	60, 180, 240 mg/kg/day for 5 days	Neg.	Lane et al. 1985
Rat (Charles River CD)	0.02-0.2% in feed	Neg.	Ellis et al. 1979
Recessive spot test			
Mouse (DBA/2J)	250 mg 2,4-tDNT/kg p.o. or i.p.	Neg.	Soares and Lock 1980

a. i.p. = intraperitoneal.
b. p.o. = per os.

levels. Since neither the 2,4- or the 2,6- isomer gave positive results with strain TA100NR3 (a strain lacking nitroreductase activity), the authors concluded that the mutagenic response of the DNTs depends solely on bacterial nitroreductase activity.

Couch et al. (1981) also reported a concentration-related increase in mutagenicity in strain TA98 without metabolic activation, for all isomers of DNT as well as tDNT. The same results were reported using the forward mutational assay with 8-azaguanine-resistance as a marker, although metabolic activation was not as effective in reducing mutagenicity as in the reversion assays.

Tokiwa et al. (1981) found 2,4-DNT to be weakly mutagenic in TA100 without metabolic activation, and reported that both 2,4- and 2,6-DNT were frameshift type mutagens, reverting strains TA1536, TA1537, and TA1538 to his+, but not reverting TA1535 (metabolic activation not specified).

Doran et al. (1985) found that under mild alkaline reaction conditions 2,4-DNT is converted to a complex mixture which possesses greatly increased levels of mutagenic activity in the Ames test strains TA100 and TA1538 (without metabolic activation) compared with 2,4-DNT alone. Mori et al. (1982) reported the results of their tests with 2,4-DNT metabolites on S. typhimurium strains TA98 and TA100 without metabolic activation. They found some of the urinary metabolites of 2,4-DNT (2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2,4-dinitrobenzyl alcohol, 2-amino-4-nitrobenzyl alcohol, 4-amino-2-nitrobenzyl alcohol, and 2,4-dinitrobenzaldehyde) to be weakly mutagenic for both strains. 2,4-diaminotoluene, 2-nitro-4-acetylaminotoluene, 2-amino-4-acetylaminotoluene, 2-amino-4-acetylaminobenzoic acid, and 2,4-dinitrobenzoic acid were found to be inactive.

Couch et al. (1980) attempted to determine whether oxidative or reductive metabolism was responsible for the formation of genotoxic products. S. typhimurium strain TA98 was used with and without metabolic activation. On a molar basis, 2-amino-4-nitrotoluene and 2-nitro-4-aminotoluene were up to 20 times as effective a mutagen as 2,4-DNT without activation. 2,4-dinitrobenzyl alcohol was 2-3 times as effective as 2,4-DNT in producing mutants in TA98. In the presence of metabolic activation, 2-amino-4-nitrobenzoic acid was found to be more mutagenic to TA98 than 2,4-DNT, and 2,4-diaminotoluene was found to be equally as effective a mutagen as 2,4-DNT. The authors concluded that the aminonitro compounds were the most potent mutagens of the metabolites studied, suggesting that reduction was the most important metabolic process producing cytotoxicity from 2,4-DNT.

Dilley et al. (1979) studied the mutagenic potential of nitrotoluene analogs found in samples of condensate water formed during purification of TNT. Although 2,4-DNT was found to have a relatively low mutagenic potency as detected by the Salmonella strain TA100 without metabolic activation, it hypothetically contributes about 13 percent to the mutagenicity of condensate water based on its prevalence in the mixture (43.4 percent).

2,4- and 2,6-DNT were tested in the Ames Salmonella assay with metabolic activation, and with the yeast Saccharomyces cerevisiae D3, before and after chlorination and ozonation (Simmon et al. 1977). 2,4-DNT was not mutagenic in any of the strains tested, with or without activation, before or after chlorination or ozonation. However, the authors note that very low concentrations of the test compounds were studied, and caution that these tests should not be considered proof that the compounds are not mutagenic. 2,6-DNT was not mutagenic on Salmonella typhimurium before or after ozonation, and it was not mutagenic on Saccharomyces cerevisiae D3 before or after chlorination or ozonation. It showed mild mutagenic activity in Salmonella typhimurium after chlorination, with a mutagenic dose response evident.

2,6-DNT was tested in the Ames Salmonella assay at concentrations of 100, 500, and 1000 μg/plate (Simmon et al. 1977). A positive response was seen in Salmonella typhimurium strains TA1537, TA1538, TA98, and TA100, with or without metabolic activation, but not in TA1535. The number of TA100 revertants per plate exhibited a discrete dose-response curve.

4.4.2 Cell Cultures

The fish cell lines RTG2 and BF2 were used to study the genotoxicity of several priority pollutants, including 2,4-DNT (Stahl and Stark 1985). Cells were examined for 96-hr cytotoxicity, anaphase-telophase aberrations, and aneuploidy/cell-cycle disruptions. The authors report that all of the chemicals tested were cytotoxic at 10 to 25 μg/mL to both cell lines, but they do not give the specific 2,4-DNT concentration tested; 2,4-DNT was also found to cause chromosome aberrations and aneuploidy/cell-cycle disruptions.

A rat hepatocyte assay using alkaline elution to detect DNA single-strand breaks was developed by Sina et al. (1983). Freshly isolated rat hepatocytes were exposed to 0.03, 0.3, 1, or 3 mmoles 2,4-DNT or 0.03, 0.3, or 3 mmoles 2,6-DNT for 3 hr with a negative elution rate (<3.0-fold over controls) reported for all concentrations but 3 mmoles. At this highest concentration tested, 2,4-DNT produced an elution rate 5.1-7.0 times that recorded for controls, and 2,6-DNT produced a rate >7.0 times that for controls, indicating an increase in DNA single-strand break frequency. A test for cytotoxicity using trypan blue dye exclusion as an indicator showed only 58 percent hepatocyte viability in cells exposed to a concentration of 3 mmoles 2,4-DNT compared with controls. At 3 mmoles 2,6-DNT, and for all other concentrations tested, greater than 80 percent viability was reported.

Pure 2,4-DNT was found to produce a significant ($p = 0.05$) dose-related increase in mutation frequency and decrease in survival in the P388 mouse lymphoma assay without the addition of metabolic activation (Styles and Cross 1983). When an activation system was included (S9 mix), there was dose-related toxicity and a slight, but not significant, increase of mutation frequency. Technical grade DNT and 2,6-DNT caused

dose-related decreases in survival but no induction of mutation with or without metabolic activation.

Chinese hamster ovary (CHO) cells were exposed to 1000 g/mL 2,6-DNT for 20 hr and assayed for protein and DNA synthesis, ATP, cell number, and viability (Garrett and Lewtas 1983). The authors report 68 percent viability and values of 25, 3, and 15 percent of controls for ATP, DNA, and protein synthesis, respectively. An estimate of 130 or 266 μg/mL was made of the concentration of 2,6-DNT that was necessary to produce a 50 percent reduction in DNA or protein synthesis, respectively. This was considered to be in the range of low toxicity (100 to 1000 μg/mL).

Couch et al. (1979) studied the influence of activation systems on the mutagenicity of 2,4-DNT to CHO cells. They found that 2,4-DNT was not mutagenic in the CHO/hypoxanthine guanine phosphoribosyl transferase (HGPRT) system using an S-9 activation system; however, lowering the oxygen tension enhanced the toxicity and mutagenicity of 2,4-DNT.

Metabolic cooperation between co-cultivated 6-thioguanine-sensitive (TGS) and 6-thioguanine-resistant (TGR) Chinese hamster lung V79 cells has been shown to be inhibited by tumor promotors, increasing the relative recovery of TGR cells compared with TGS cells. The V79 cell metabolic cooperation assay for tumor promotors was used to test tDNT, 2,4-DNT, and 2,6-DNT (Dorman and Boreiko 1983). Concentrations of from 10^{-3} to 10^{-9} M of each of the three compounds did not markedly increase the recovery of TGR cells, indicating the absence of tumorigenic potential. However, the authors state that this may be due partially to the limited capability for xenobiotic transformation inherent in the cell lines studied, and to the fact that in vivo metabolism of the DNTs proceeds by numerous steps, difficult to reproduce in in vitro studies.

Technical grade dinitrotoluene and several of the pure isomers were tested for mutagenicity using the CHO/HGPRT somatic cell mutagenesis assay (Abernathy and Couch 1982). In this assay, compounds are tested for their ability to induce mutation to 6-thioguanine resistance in the presence or absence of metabolic activation with the microsomal mixed function oxidase. Although the isomers containing nitro groups on adjacent carbon atoms (2,3- and 3,4-DNT) greatly decreased survival, in the absence of metabolic activation none of the isomers or the tDNT was found to increase the thioguanine-resistant fraction of surviving cells with or without activation.

Bermudez et al. (1979) found 2,6-DNT to elicit a negative response in the Fischer 344 rat primary hepatocyte unscheduled DNA synthesis (UDS) assay. 2,4-diaminotoluene (2,4DAT) gave a positive response. Similar results were reported for 2,6-DNT and 2,4DAT in cultures of primary human hepatocytes (Butterworth et al. 1983). An in vitro spermatocyte DNA repair assay also found that 2,6-DNT failed to induce UDS in Charles River CD rats (Working and Butterworth 1984).

4.4.3 In Vivo-In Vitro DNA Repair

Since in vitro systems alone may not reflect the activation of compounds that may occur in the whole animal, Mirsalis, Tyson, and Butterworth (1982) used an in vivo-in vitro hepatocyte DNA repair assay to investigate the genotoxicity of 2,4- and 2,6-DNT, and 2,4- and 2,6-diaminotoluene (2,6DAT). All compounds but 2,6DAT were found to produce strong genotoxic responses using male Fischer 344 rats, although the 2,4- isomer was an order of magnitude less genotoxic than the 2,6-isomer.

Mirsalis and Butterworth (1982) observed a dose-related increase in UDS following in vivo treatment of male Fischer 344 rats with 200 mg/kg tDNT. They also noted a 50-fold increase in the number of cells in the S-phase of interphase at 48 hr after treatment. 2,4-DNT was found to elicit a weak UDS response at an in vivo dose of 100 mg/kg, while 2,6-DNT proved lethal to cells in culture at this in vivo dose. An in vivo dose of 5 mg/kg 2,6-DNT produced the same UDS response as the dose of 100 mg/kg 2,4-DNT, while 20 mg/kg of the 2,6- isomer induced a response similar to 100 mg/kg tDNT. Female Fischer 344 rats treated with tDNT showed a much lower level of UDS than male rats, with only a slight increase in the number of cells in S-phase relative to males.

Mirsalis et al. (1982) used the in vivo-in vitro hepatocyte DNA repair assay in an effort to test the theory that gut microflora play an important role in the genotoxicity of DNT. They found extensive UDS in hepatocytes of conventional male Fischer 344 rats treated with DNT (isomer unspecified) and a negative UDS response in axenic rats treated similarly.

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

A National Institute for Occupational Safety and Health (NIOSH) health evaluation study suggested the possibility of adverse reproductive effects in male workers exposed to DNT and toluene diamine (TDA) (Ahrenholz and Channing 1980). DNT concentrations in workroom air were found to range from 0.013 to 0.42 mg/m^3 during the initial survey, and from nondetectable to 0.10 mg/m^3 during a follow-up survey. All DNT air concentrations were below the OSHA standard of 1.5 mg/m^3. The study addressed miscarriages, abnormal offspring, and semen abnormalities (none of which could be conclusively related to DNT exposures) and suggested that DNT may adversely affect the male reproductive system. Medical findings included a significant reduction in sperm count among exposed workers when compared to controls; however, this significant reduction in sperm count reported in the study has been questioned by Hamill et al. (1982), who felt that the control groups had an abnormally high sperm count.

In a survey designed to corroborate the above NIOSH findings, 78 men working in another DNT/TDA chemical plant (83 percent of the exposed population) participated in an epidemiological assessment of reproductive hazard (Hamill et al. 1982). Based on reproductive history and

clinical results, Hamill et al. (1982) concluded that no detectable reproductive effects existed among the male workers exposed to DNT and TDA.

In the studies of McGown et al. (1983), male and female Sprague-Dawley rats were fed 0, 0.9, 1.2, 1.9, or 3.0 g/kg 2,4-DNT in the diet (representing 45, 60, 94, or 143 mg/kg/day, respectively) for 14 days. A dose-dependent oligospermy with degenerative changes in testes of male rats was reported.

Soares and Lock (1980) report a marked reduction in the percent of fertile matings following oral or i.p. administration of 250 mg/kg of pure 2,4-DNT or tDNT (two consecutive treatments) to male DBA/2J mice mated with CD-1 female mice.

Smith (1984) assayed 2,4-DNT for its potential to produce adverse reproductive effects in CD-1 female mice. After estimating a maximum tolerated dose, 50 timed-pregnant female mice were administered 390 mg/kg/day during gestational days 7 and 14. Ten of the dosed females died during the dosing period, and five more died during the post-dosing observation period. Of these 15, six females were found to be pregnant. Five additional litters were found to be reabsorbed. A reproductive index (RI) of 0.68 was reported (significantly different from controls at $p = 0.01$). This low RI was partially attributed to the high maternal toxicity. Other measures of reproductive toxicity were not significantly different from controls, although there was a slight trend toward lower maternal weight gains during pregnancy.

Timed-pregnant Fischer 344 albino rats were administered tDNT by gavage (76 percent 2,4-DNT and 19 percent 2,6-DNT) mixed in corn oil on gestational days 7 through 20 (Price et al. 1985). Study groups included a vehicle control, a hydroxyurea positive control, and dosage groups receiving 14, 35, 37.5, 75, 100, or 150 mg/kg/day of tDNT. During the treatment period, 46.2 percent of the pregnant females exposed to 150 mg/kg/day died between gestational day 11 and 18. One rat died in each of the 14, 35, and 100 mg/kg/day groups, but the authors felt that improper intubation might have been the cause. Treatment-related increases in maternal relative liver and spleen weight as well as a dose-related decrease in absolute maternal weight gain were seen across all treatment groups during the gestation period. However, no statistically significant ($p = 0.05$) effects on percent resorptions or percent dead or alive fetuses were seen. A noticeable increase in percent resorptions and dead fetuses was seen at the 150 mg/kg/day dose (49.6 percent as compared with 16.8 percent for vehicle controls), but this increase was not statistically significant, due to the small number of litters. In addition, there were no statistical differences in sex proportions, average fetal body weight, average fetal length, or average placental weight per litter. Dams and fetuses from the 100 mg/kg/day group exhibited typical hematological signs of DNT toxicity (reticulocytosis, decreased RBC count, increased RBC size, etc.).

A three-generation reproductive study in Charles River CD rats found no specific reproductive effects of 2,4-DNT from daily intakes of

0.57, 3.9, and 34 mg/kg/day in males and 0.71, 5.1, and 45 mg/kg/day in females (Ellis et al. 1979). However, the combined effects of the overall toxicity of 2,4-DNT in the high dose group (decreased body weight and aspermatogenesis) resulted in the absence of an F_2 parental generation, and only few matings in the F_1, indicating an adverse effect on reproductive performance. 2,4-DNT appeared to increase the occurence of deaths during parturition, associated with a prolonged parturition, excessive hemorrhaging, or retention of placentas or fetal-placental units. This observed adverse reproductive effect is attributed by the authors to 2,4-DNT toxicity.

The results of dominant lethal assays in rats are given in Table 16. In the study by Lane et al. (1985), the highest dose of 2,4-DNT (240 mg/kg/day) resulted in a reduced number of sperm-positive and pregnant females; these effects diminished in the later weeks of mating. There were no consistent changes seen in numbers of pre-implantation losses, implantation sites, or living and dead fetuses at 60 or 180 mg/kg/day. Statistical evaluations at the high dose were impossible, due to the 53 percent mortality seen at this level. There was a dose-dependent decrease in body weight gain seen in the rats. The results indicated that 2,4-DNT adversely affects reproductive performance, but does not cause a dominant lethal effect.

Chronic feeding studies with mice fed 13.5, 95, or 900 mg/kg/day 2,4-DNT resulted in decreased testis weight, aspermatogenesis, and atrophy of the testes in most high-dose and almost half of mid-dose males; also observed were nonfunctioning follicles, lacking corpora lutea, in 63 percent of high-dose females (Ellis et al. 1979). Similar results were seen in rats fed 35 mg/kg/day 2,4-DNT for 2 yr (Ellis et al. 1979), with almost all high-dose males exhibiting severe atrophy of the seminiferous tubules and almost complete lack of spermatogenesis. The incidence of testicular atrophy was about 29 to 33 percent in lower dose males, compared with 16 percent in control males.

Ellis et al. (1979) reported a dose-related decrease in weight gain and fertility, and a dose-related increase in spermless vaginal plugs during their dominant lethal mutation studies. In these studies, they administered 0.57, 3.9, or 34 mg/kg/day 2,4-DNT in feed to male Charles River CD rats for 13 weeks.

4.6 ONCOGENICITY

An 18-month study of the carcinogenicity of >95 percent pure 2,4-DNT was performed using Fischer 344 rats and B6C3F1 mice by the National Cancer Institute (NCI 1978). Groups of 50 male and 50 female rats were fed 0.02 or 0.008 percent 2,4-DNT in the diet daily for 18 months, with observation continuing for an additional 6 months. The high dose resulted in an approximate total intake of 14 mg/kg/day, of which 13.3 mg/kg/day was 2,4-DNT and 0.7 mg/kg/day was 2,6-DNT. In male rats, a significant increase in the incidence of fibroma of the skin and subcutaneous tissue occurred at the high ($p = 0.003$) and low ($p = 0.008$) doses. A slight, but not statistically significant, increase

(6 percent) in the number of hepatocellular carcinomas was seen in the high-dose male rats, compared with none observed in control rats. A statistically significant ($p = 0.016$) incidence of fibroadenoma of the mammary gland occurred in the high-dose female rats. No evidence of treatment-related tumors was seen in male or female mice at either dose tested.

In the studies by Ellis et al. (1979), in which beagle dogs, rats, and mice were fed 98 percent pure 2,4-DNT for 2 yr (Sect. 4.3.2.2), no evidence of carcinogenicity was seen in dogs. The most significant tissue lesion reported in the Charles River CD rat was the progressive development of hepatocellular carcinoma, seen in the early stages (foci of altered hepatocytes) after 12 months dosing at all concentrations, and seen in more advanced stages (hepatic neoplastic nodules and hepatocellular carcinoma) between 1 and 2 yr at the mid- and high-dose levels. A summary of the hepatic lesions reported in both sexes of rats after 2-yr treatment is given in Table 17. The incidence of hepatocellular carcinomas in male rats at the high dose level was not significantly different from control rats. However, only four of the high-dose males remained alive after week 92 of the study, and all of the high-dose males had died by week 97. Hepatocellular carcinomas were identified in 11 of the 14 female rats surviving after week 92. In addition to the hepatic tumors evident in both sexes of rats as in the NCI (1978) study discussed above, there was a greatly increased incidence of subcutaneous tumors; in male rats these were mostly fibromas, and in female rats, these were mammary fibroadenomas. It can be estimated that of the 34 mg/kg/day of 98 percent pure 2,4-DNT administered to male rats, 33.3 mg/kg/day was 2,4-DNT, and 0.7 mg/kg/day was the 2,6- isomer; in the female rat study, approximately 44.1 mg/kg/day was 2,4-DNT and 0.9 mg/kg/day was 2,6-DNT.

In the studies on the effects of 2,4-DNT in the diet of Charles River CD-1 mice (Ellis et al. 1979), there was an increased incidence and greater severity of hepatocellular dysplasia and nephropathy in high-dose (900 mg/kg/day) mice of both sexes, and then was a greater incidence of kidney tumors, including cystic papillary adenomas, solid renal cell carcinomas, and cystic papillary carcinomas, in middle-dose (95 mg/kg/day) males after 24 months of study. All of the male and 95 percent of the female mice fed the high dose died before the end of the study. Liver dysplasia and a few kidney tumors were observed in low-dose (13.5 mg/kg/day) males after 24 months.

During a 2-yr study of tDNT (containing 18.8 percent 2,6-DNT) in the diet of Fischer 344 rats, 0, 3.5, 14.0, or 35 mg/kg/day was fed to groups of 130 animals of each sex (CIIT 1982). In this study, of the 35 mg/kg/day tDNT administered, approximately 26.8 mg/kg/day was the 2,4-isomer, and 6.6 mg/kg/day was the 2,6- isomer. All living animals in the high-dose group were sacrificed at week 55, due to histopathological findings discovered earlier, but the other dose groups were carried until termination of the study. The incidence of hepatocellular carcinomas and hepatic neoplastic nodules found in all rats at the high dose level, and those living until termination of the study or found dead or moribund at the low and mid dose levels is given in Table 18. It can be

TABLE 17. INCIDENCE OF HEPATIC LESIONS IN CHARLES RIVER CD RATS FED 2,4-DNT[a,b]

Dose (mg/kg/day)	Lesion	Incidence at 24 months		Incidence in unscheduled deaths		Total HNN[c] or HCC[d]	
		Female	Male	Female	Male	Female	Male
0.0	HNN	0/7[e]	0/8	0/12	0/13		
	HCC	0/7	1/8	0/12	1/13		
						0/19	2/21
0.71[f] or 0.57[g]	HNN	1/10	0/9	1/23	0/16		
	HCC	0/10	0/9	0/23	2/16		
						2/33	2/25
5.1[f] or 3.9[g]	HNN	1/6	0/5	1/17	0/13		
	HCC	0/6	0/5	1/17	1/13		
						3/23	1/18
45.3[f] or 35.0[g]	HNN	0/1	-	6/34	2/30		
	HCC	1/1	-	18/34	6/30		
						23/35	8/30

a. 2,4-DNT = 2,4-dinitrotoluene.
b. From Ellis et al. 1979.
c. HNN = hepatic neoplastic nodules.
d. HCC = hepatocellular carcinomas.
e. Represents the number of animals with lesions/number of animals examined.
f. Dose to female rats.
g. Dose to male rats.

TABLE 18. INCIDENCE OF HEPATIC LESIONS IN FISCHER 344 RATS FED tDNT FOR 104 WEEKS[a,b]

Dose (mg/kg/day)	Lesion	Incidence at 104 weeks		Incidence in unscheduled deaths		Total HNN[c] or HCC[d]	
		Male	Female	Male	Female	Male	Female
0.0	HNN	9/61[e]	5/57	0/19	1/23		
	HCC	1/61	0/57	0/19	0/23		
						10/80	6/80
3.5	HNN	11/70	12/61	3/20	1/29		
	HCC	9/70	0/61	1/20	1/29		
						23/90	14/90
14.0	HNN	16/23	53/69	31/65	12/22		
	HCC	22/23	41/68	57/65	1/22		
						83/88	79/90
35.0	HNN	5/20	12/20	-	-		
	HCC	20/20	11/20	-	-		
						20/20	19/20

a. t-DNT = technical grade dinitrotoluene.
b. From CIIT 1982.
c. HNN = hepatic neoplastic nodules.
d. HCC = hepatocellular carcinomas.
e. Represents the number of animals with lesions/number of animals examined.

seen that dietary intake of tDNT resulted in hepatocellular carcinomas in 20/20 high-dose male rats and 11/20 high-dose female rats after 1 yr, and it resulted in 22/23 mid-dose male rats and 41/68 mid-dose female rats after 2 yr. An increased incidence of hepatic neoplastic nodules was seen after 1 yr treatment in rats of both sexes at the high dose, and after 2 yr in low- and mid-dose female rats and mid-dose male rats. Also reported was an increased incidence of other benign tumors, including mammary fibroadenomas in low-dose males and mid-dose rats of both sexes, and subcutaneous fibromas in males at both doses (not shown on Table 18). The presence of several cholangiocarcinomas seen in males at the mid- and high-doses was assumed to be treatment-related.

Groups of 28 male Fischer 344 rats were fed either pure 2,4-DNT (27 mg/kg/day), tDNT (35 mg/kg/day), or pure 2,6-DNT (7 or 14 mg/kg/day) for a year (Leonard et al. 1987). Controls were fed a normal diet. Phenobarbital-fed rats were included as a promoter control. The tDNT was 18.8 percent 2,6-DNT, representing approximately 6.6 mg/kg/day 2,6-DNT. Hepatic microsomes and cytosol were prepared from eight animals from each group during the study period. Table 19 gives the results for the animals remaining at termination of the study. Animals with hepatic neoplastic nodules contituted 5, 53, 90, and 79 percent, respectively, of the four DNT dose groups. Animals with only hepatocarcinomas represented 0, 47, 85, and 100 percent, respectively, of the four DNT dose groups. All of the rats fed 7 or 14 mg/kg/day 2,6-DNT had either hepatic neoplastic nodules or hepatic carcinomas. Multiple hepatic carcinomas were frequently found in the tDNT and 2,6-DNT groups, and 10 percent of the animals treated with tDNT or 7 mg/kg/day 2,6-DNT had cholangiocarcinomas. None of the phenobarbital-fed rats exhibited hepatic neoplastic nodules and hepatic carcinomas.

TABLE 19. INCIDENCE OF HEPATIC LESIONS IN MALE FISCHER 344 RATS FED 2,4-DNT[a], tDNT[b], OR 2,6-DNT[c] FOR 52 WEEKS[d]

Lesion	Treatment Group				
	Control	2,4-DNT	tDNT	2,6-DNT	2,6-DNT
	0.0[e]	27[e]	35[e]	7[e]	14[e]
HNN[f]	0/20[g]	1/20	10/19	18/20	15/19
HCN[h]	0/20	0/20	9/19	17/20	19/19

a. 2,4-DNT = pure 2,4-dinitrotoluene.
b. t-DNT = technical grade dinitrotoluene containing 18.8 percent 2,6-DNT.
c. 2,6-DNT = pure 2,6-dinitrotoluene.
d. From Leonard et al. 1987.
e. Dose in mg/kg/day.
f. HNN = hepatic neoplastic nodules.
g. Represents the number of animals with lesions/number of animals examined.
h. HCC = hepatocellular carcinomas.

Metastatic hepatocellular carcinomas were reported in 58 and 15 percent, respectively, of the high- and low-dose 2,6-DNT animals. Although no isomer-specific or dose-dependent effects were seen, hepatocyte degeneration and vacuolization were reported in the majority of animals fed DNT. Acidophilic and basophilic cell foci were exhibited in over 90 percent of the animals fed 2,6-DNT or tDNT, while 70 percent of the rats fed 2,4-DNT had acidophilic foci and 10 percent had basophilic foci. These changes were only occasionally noted in either control group. The authors note that the occurrence of basophilic foci parallels the occurrence of hepatic carcinomas in the study groups, and might more closely predict carcinogenesis than the acidophilic foci.

Hepatic microsomal epoxide hydrolase (EH) and cytosolic DT-diaphorase (DTD) were used as phenotypic markers of neoplastic nodules. After 6 months of feeding, EH activity was significantly ($p = 0.05$) elevated in the 2,4-DNT fed rats (about 220 percent of control values) as well as in the 2,6-DNT fed rats (about 380 and 480 percent, respectively, at the low and high dose). Phenobarbital-fed rats showed an increase in EH activity of 400 percent of controls. By 12 months, EH activity had declined in all groups, but still remained higher than in controls. Activity of DTD was similar to controls in the 2,4-DNT or phenobarbital-fed rats throughout the study period, but it increased with time and dose for the 2,6-DNT fed rats, representing 420 and 650 percent of controls after 1-yr feeding of 7 or 14 mg/kg/day 2,6-DNT, respectively.

The authors conclude from this study that 2,6-DNT is a complete hepatocarcinogen, and that most if not all of the carcinogenicity of tDNT can be attributed to the 2,6- isomer. The data suggest that 2,4-DNT is not a carcinogen and may reduce the carcinogenicity of 2,6-DNT when administered with it. However, failure to test more than one dose level for 2,4-DNT, testing of only male rats, and testing for only 1 yr preclude the use of this study for a definitive statement regarding the carcinogenicity of pure 2,4-DNT. Using the USEPA (1984) weight-of-evidence classification for carcinogenicity, 2,4-DNT is best considered to be in Group D. That is, because of major qualitative or quantitative limitations, the study described above (Leonard et al. 1987) cannot be interpreted as showing either the presence or absence of a carcinogenic effect for 2,4-DNT.

The effect of dietary pectin on microfloral metabolic activity and the subsequent induction of hepatic focal lesions and tumors by 2,6-DNT was studied in male Fischer 344 rats (Goldsworthy et al. 1986). A cereal-based diet with about 5 percent pectin (NIH-07), a purified pectin-free diet (AIN-76A), and the purified diet supplemented with 5 percent pectin (AP) were administered for 1 yr. Each diet contained 0 (controls), 0.6 to 0.7, or 3.0 to 3.5 mg/kg/day 2,6-DNT. Monthly feed consumption and mortality rates did not differ between the groups. Multiple gross lesions resulted in elevated liver weights in the high-dose NIH group. Dose- and time-dependent increases in the number of animals with hepatic foci (as measured by staining for gamma-glutamyl transpeptidase) were seen in all groups, regardless of diet. However, no tumors were observed in any of the rats fed control diets or AIN and AP diets

at either dose level. All of the high-dose NIH-treated animals had either hepatic neoplastic nodules or hepatic carcinomas after 1 yr, and 5 of 10 had two or more lesions. Three rats fed the low-dose NIH diet had neoplastic nodules. The authors were unable to explain the absence of any hepatic foci or tumors in the rats fed 2,6-DNT in the purified diet with 5 percent pectin, but they hypothesize that something other than pectin may be contributing to the 2,6-DNT-induced hepatocarcinogenesis. They suggest that the influence of exogenous agents in the cereal-based diet on foci and tumor induction in the rat liver may have implications in the interpretation of carcinogenicity bioassays and in the comparison of data between laboratories.

In an effort to explain the conflicting results reported in the literature regarding the carcinogenicity of various isomers of DNT (NCI 1978; Ellis et al. 1979; CIIT 1982), Leonard et al. (1986, 1983, 1982) have compared the induction of hepatic tumors by the DNT isomers and tDNT using hepatic initiation-promotion tests.

The appearance of gamma glutamyl transpeptidase positive (GGT+) foci represents an endpoint for in vivo hepatic tumor initiation-promotion studies. Using this endpoint, Leonard et al. (1983) found that tDNT was an initiator when administered as a single dose of 75 mg/kg to male Fischer 344 rats 12 hr following a partial hepatectomy. Purified DNT isomers (2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT) were also tested using hepatic initiation-promotion protocols, and only 2,6-DNT was found to significantly ($p = 0.05$) increase the number of GGT+ foci compared with controls. A dose-dependent response in number of GGT+ foci was seen with doses of 2,6-DNT from 37.5 to 150 mg/kg. Although both tDNT and 2,6-DNT were considered to be weak initiators, the authors concluded that the initiating capacity of the tDNT was due to the presence of the 2,6-DNT isomer alone.

In a study designed to investigate the tumor promoting activity of DNT, Leonard et al. (1982) initiated male Fischer 344 rats with a single dose of diethylnitrosamine (DEN) or saline (controls) prior to feeding either 27 mg/kg/day 2,4-DNT (>99.4 percent pure) or 10 mg/kg/day 2,6-DNT for 12 weeks. The presence of GGT+ foci relative to controls was used as the endpoint, and 2,4-DNT was found to moderately increase the number when fed to DEN-initiated animals, but not when fed to controls. Therefore, 2,4-DNT has hepatic-tumor promoting activity, but not initiating activity. However, 2,6-DNT was found to have relatively potent foci enhancing activity with, and moderate activity without, DEN initiation, indicating that it has both initiating and promoting capabilities.

Based on the results of the above studies, Leonard et al. (1986) conclude that 2,6-DNT is a potent hepatocarcinogen with both hepatic-tumor initiating and promoting activity, while 2,4-DNT is an apparent pure promoter. In addition, the carcinogenicity of tDNT is largely accounted for by the presence of the 2,6- isomer. These results provide an explanation for the discrepancies seen in the results of carcinogenicity testing with DNT isomers.

Administration of 10 mg/kg/day 2,4-DNT to beagle dogs for 2 yr resulted in no morphological aberrations of the chromosomes in kidney or bone marrow cultures; chromosome analysis of bone marrow and kidney cultures from rats fed 3.9 or 35 mg/kg/day 2,4-DNT for 2 yr showed the same results (Ellis et al. 1979).

Schut et al. (1982), Stoner et al. (1984), and Maronpot et al. (1983) reported negative results for the pulmonary tumor bioassay following administration of 2,4-DNT to Strain A/J mice. Maronpot et al. (1983) also reported negative results in the pulmonary tumor assay using male Strain A/ST mice, but found positive results using female A/ST mice. Slaga et al. (1985) reported that neither 2,4-DNT, 2,6-DNT nor a 2:1 mixture of 2,4- and 2,6-DNT gave an unequivocal positive response in the lung-tumor assay test using Strain A/J mice.

Young, adult, Strain A/J mice were injected i.p. three times weekly for 8 weeks with a total of 600, 1500, or 3000 mg/kg 2,6-DNT or dosed by gavage with 1200, 3000, or 6000 mg/kg 2,6-DNT on the same schedule (Stoner et al. 1984). All mice were killed 22 weeks after the last injection, and lungs were removed and examined for tumors. Control groups consisted of untreated, vehicle (tricaprylin)-treated, and urethane-treated animals. The average number of lung tumors per mouse in 2,6-DNT treated mice was not significantly different ($p > 0.05$) from untreated and vehicle treated controls. The lung tumor response to the positive control (urethane treated) was significantly ($p < 0.05$) increased when compared with the other controls. Similar results were reported for a 2:1 mixture of 2,4-DNT and 2,6-DNT administered as 1200, 3000, or 6000 mg/kg by gavage or 960, 2400, or 4800 mg/kg i.p.

4.7 SUMMARY

Both 2,4- and 2,6-DNT are rapidly absorbed from the intestine, with absorption of the 2,6- isomer being slightly slower. Intestinal absorption of 2,4-DNT in the mouse appears to occur to a lesser extent than in the rat. Metabolites excreted in the bile are subsequently reabsorbed from the intestines. Inhalation and skin absorption of the DNT isomers may also occur. In the rat, there are no sex differences in the concentration or elimination of DNT metabolites from plasma or kidneys or in elimination from the liver; however, metabolites are predominantly found covalently bound to hepatic macromolecules, and the concentration of metabolites is higher in livers of male rats than in livers of female rats. Hepatic binding of 2,6-DNT in protein, RNA, and DNA is 2 to 5 times greater than that of 2,4-DNT.

2,4-DNT absorbed from the intestine of laboratory animals is principally metabolized by the liver to dinitrobenzyl alcohol (DNBAlc), which is conjugated to form dinitrobenzyl alcohol glucuronide (DNBAlcG). In female rats, the DNBAlcG is primarily excreted in the urine, while in male rats biliary excretion of DNBAlcG predominates. In males or females, the DNBAlcG that is excreted in the bile returns to the intestine, where it is deconjugated by intestinal microflora to DNBAlc. This DNBAlc may then be further metabolized by the microflora to an active

metabolite, which is absorbed once again and is covalently bound to hepatic macromolecules. The principal route of excretion of 2,4- and 2,6-DNT and their metabolites is via the urine, with excretion virtually complete after 24 hr.

In humans, there is no substantial evidence of accumulation of 2,4- or 2,6-metabolites from day to day, and no apparent difference in the time course of elimination of metabolites. The presence of 2-amino-4-nitrobenzoic acid and 2-acetylamino-4-nitrobenenzoic acid as major metabolites in human urine indicates that nitro group reduction is occurring in humans. Although the metabolites identified in human urine are qualitatively similar to those identified in rat urine, there are quantitative differences in the metabolites measured. In male humans, over half (52.5 percent) of total metabolites excreted are 2,4- and 2,6-dinitrobenzoic acid (DNBA), 37 percent are 2-amino-4-nitrobenzoic acid (2A4NBA), and 9.5 percent are 2,4- or 2,6-dintrobenzyl alcohol glucuronide (DNBAlcG). In male rats, about 50 percent of the urinary metabolites were identified as DNBA, but only 5 percent were identified as 2A4NBA. In female humans, 28.8 percent of the metabolites excreted are DNBA and 33.3 percent are DNBAlcG.

Rats appear to be more susceptible to 2,4-DNT toxicity than mice, with oral LD_{50} values for rats and mice ranging from 268 to 650 mg/kg and 1250 to 1954 mg/kg, respectively. Male rats are more sensitive to 2,4-DNT than female rats. Male rats are also more sensitive to 2,6-DNT than 2,4-DNT toxicity, while female rats are more tolerant. Both sexes of mice are more sensitive to the 2,6- isomer than the 2,4- isomer, with oral LD_{50} values for both sexes ranging from 621 to 1000 mg/kg. Toxic signs for both isomers are central nervous system depression resulting in ataxia, respiratory depression, and death within 24 hr. 2,4-DNT was found to induce methemoglobinemia and sulfhemoglobinemia in rats, with the presence of Heinz bodies and reticulosis.

Subchronic feeding studies with 2,4-DNT were lethal in 50 percent of beagle dogs fed 25 mg/kg/day, with neuromuscular effects, methemoglobinemia with Heinz bodies, anemia, reticulocytosis, extramedullary hematopoiesis, and reduced spermatogenesis. A dose of 5 mg/kg/day 2,4-DNT is reported as a no observed effects level (NOEL). Doses of 20 mg/kg/day 2,6-DNT resulted in 25 percent lethality in dogs, and clinical signs similar to those from 2,4-DNT, including lesions in the liver and spleen. A dose of 5 mg/kg/day 2,6-DNT caused mild splenic hematopoiesis. No NOEL was reported for 2,6-DNT in dogs.

Rats exhibited the same clinical signs as a result of 13 weeks exposure to 2,4-DNT toxicity in dogs, with a lowest observed adverse effects level (LOAEL) of 34 to 38 mg/kg/day reported. severe testicular atrophy and aspermatogenesis were reported at 45 mg/kg/day. An NOEL of 7.2 to 7.4 mg/kg/day 2,4-DNT is reported for rats, with 34 mg/kg/day and higher resulting in clinical signs similar to those seen with the 2,4- isomer, as well as testicular, bile duct, and splenic lesions.

Mice are more tolerant of subchronic 2,4-DNT toxicity than dogs or rats, with an NOEL of 13.7 to 14.7 mg/kg/day and with only mild anemia

and weight loss seen at 413 to 468 mg/kg/day. However, as in dogs and rats, they seem to be more susceptible to 2,6-DNT toxicity, with an NOEL of 11.0 mg/kg/day for both sexes, and with testicular atrophy, bile duct hyperplasia, and liver and splenic degenerative changes seen at 50 to 55 mg/kg/day. Doses of 50 or 290 mg/kg/day resulted in 100 percent death of males and 12 and 75 percent death, respectively, of females.

Subchronic and chronic exposures of workers in munitions plants have been reported from the early and mid-1900s. Symptoms recorded following exposure were cyanosis, dizziness, and a tendency to sleep, with headache, dyspnea, and brown urine. Alcoholic subjects were quite sensitive to toxicity. Aching in joints, particularly the knees, was reported, continuing in some cases for several months after exposure. Recovery occurred 2 to 3 days after exposure, and more quickly if the subject left the area immediately. Other symptoms included vomiting, numbness and tingling, loss of weight, and diarrhea. No fatalities were recorded. Improvements in hygiene standards have nearly eliminated the acute effects of DNT exposures in modern industry.

A study of cohorts of workers exposed to DNT in two munitions plants operating in the 1940s and 1950s indicated an increase in ischemic heart disease over that seen in white males in the United States and in persons living in the vicinity of the two plants. The data suggest a correlation between mortality and length and intensity of exposure to DNT.

Significant reductions in body weight gain and increases in liver weight were seen in male Fischer 344 rats following administration of 27 mg/kg/day 2,4-DNT, 35 mg/kg/day tDNT, or 7 or 14 mg/kg/day 2,6-DNT for 1 yr. Serum alanine aminotransferase was elevated following administration for 52 weeks of both doses of 2,6-DNT, and serum gamma-glutamyl transferase was increased after 26 and 52 weeks at the high dose of 2,6-DNT. Neither parameter was affected by 2,4- or tDNT.

Male and female Charles River CD rats fed 0.57 (males) and 0.71 (females) mg/kg/day 2,4-DNT for 2 yr showed no apparent effects, while rats fed 3.9 and 5.1 mg/kg/day showed some mild effects in susceptible individuals. Severely decreased weight gain, shortened life span, and various pathological effects were seen at 34 and 45 mg/kg/day for male and female rats, respectively. The three major causes of death were pituitary tumors, ulcerated subcutaneous tumors, and loss of vigor. The most significant tissue lesion was the progressive development of hepatocellular carcinoma seen in advanced stages after 2 yr at the mid- and high dose levels. All of the high-dose rats but one had died before the end of month 23, with only a few more deaths seen in the middle-dose group than in controls.

In chronic toxicity studies of 2,4 DNT in male and female Charles River CD 1 mice, all of the male mice fed the high dose (900 mg/kg/day) died by month 18 of the study, and about 95 percent of the female mice fed the high dose died by month 21 of the study. After 1 yr, most of these mice exhibited anemia, with decreased erythrocytes and hemoglobin. A high level of Heinz bodies, increased reticulocytes, and occasional

methemoglobinemia was seen. Similar effects were seen in some moribund mice dosed at 95 mg/kg/day. Kidney tumors appeared after 2 yr in surviving males. Aspermatogenesis and atrophy of the testes were observed in almost all middle- and high-dose males, and nonfunctioning follicles, lacking corpora lutea, were seen in 63 percent of high-dose females. A dose- and time-dependent increase in pigmentation, particularly of the spleen and liver, was reported for mice at all levels. There were an increased incidence and greater severity of hepatocellular dysplasia and nephropathy in high-dose mice, and a greater incidence of kidney tumors in middle-dose males after 24 months of study. Liver dysplasia and a few kidney tumors were observed in low-dose males.

The genotoxicity of 2,4- and 2,6-DNT has been studied by various authors using a multitude of short-term toxicity assays. A weak mutagenicity has been reported for the 2,4- isomer using the Ames Salmonella assay, with reduction or elimination of activity in the presence of a metabolic activation system. The 2,6- isomer gave a positive response in the Ames assay with or without metabolic activation. Neither the 2,4- nor the 2,6- isomer gave a positive result using a Salmonella strain lacking nitroreductase activity, indicating that the mutagenic response of the DNTs depends solely on bacterial nitroreductase activity. Further, the reductive metabolites of 2,4-DNT were found to be up to 20 times as effective in producing mutations using the Ames assay, whereas the acetylamino toluenes and 2,4-diaminotoluene were not mutagenic.

2,4-DNT was found to cause chromosome aberrations and aneuploidy/cell-cycle disruptions in the RTG2 and BF2 fish cell lines, and it caused an increase in DNA single-strand break frequency using a rat hepatocyte assay. Pure 2,4-DNT was found to produce a dose-related increase in mutation frequency and a decrease in survival in the P388 mouse lymphoma assay, whereas tDNT and 2,6-DNT produced no mutagenic effect. However, the 2,6- isomer reduced DNA and protein synthesis in CHO cells. The V79 cell metabolic cooperation assay and the CHO/HGPRT somatic cell mutagenesis assay both proved negative for 2,4-, 2,6-, and tDNT.

No morphological aberrations of chromosomes were seen in kidney or bone marrow cultures following 2-yr administration of 2,4-DNT to dogs or rats. Neither 2,4- nor 2,6-DNT increased the number of lung tumors in Strain A/J mice following i.p or oral administration for 8 weeks.

Both 2,4- and 2,6-DNT were found to elicit a negative response in the in vitro UDS assay. However, in vivo-in vitro UDS assays demonstrated that both isomers produce genotoxic responses, with the 2,6- isomer an order of magnitude more toxic than the 2,4- isomer. A dose of 5 mg/kg of 2,6-DNT produced the same UDS response as a dose of 100 mg/kg of the 2,4- isomer; 20 mg/kg 2,6-DNT produced the same response as 100 mg/kg tDNT. Female rats were less responsive to UDS than male rats following intake of tDNT. In addition, conventional male rats exhibited extensive UDS and axenic rats exhibited a negative UDS response following administration of DNT. These results support the hypothesis that reductive metabolism occurring in the gut of male animals is responsible

for the cytotoxicity of the DNT isomers, and that the 2,6- isomer is responsible for the apparent cytotoxicity of tDNT.

No positive correlation was established between 2,4-DNT and reproductive effects observed among workers in a DNT factory, or reported in rat or mouse studies using 2,4- or tDNT. A high maternal and embryo/fetal toxicity was observed, however, in laboratory studies. Dominant lethal assays proved negative for 2,4-DNT.

2,4-DNT was not found to be carcinogenic in male or female Fischer 344 rats following daily intake of 0.02 or 0.008 percent 2,4-DNT in the diet for 18 months. In male rats there was a significant increase in skin and subcutaneous tissue fibromas, and in female rats there was an increase of fibroadenoma of the mammary gland. Similarly, 2,4-DNT was not found to be carcinogenic in beagle dogs following administration of up to 10 mg/kg/day for 2 yr.

However, the progressive development of hepatocellular carcinoma was reported in Charles River CD rats, with 66 percent of the females and 27 percent of the males exhibiting hepatic neoplastic nodules or hepatic carcinomas following doses of 45 and 35 mg/kg/day, respectively, for more than a year. At this high dose, all of the males and all but one of the females had died before the end of the 2-yr study. In addition to hepatic tumors, there was also a greatly increased incidence of benign subcutaneous tumors.

In 2-yr feeding studies with Charles River CD-1 mice, the high dose of 2,4-DNT (900 mg/kg/day) resulted in nearly 100 percent lethality. Although no hepatic tumors were observed, a greater incidence of kidney tumors was seen in middle-dose (95 mg/kg/day) male mice after 24 months.

During a 2-yr feeding study of tDNT in the diet of Fischer 344 rats, 100 percent of the male rats and 55 percent of the female rats had developed hepatocellular carcinomas by week 55 of the study following administration of 35 mg/kg/day. After 2 yr of feeding 14 mg/kg/day tDNT, hepatic carcinomas were observed in 96 percent of surviving males and 60 percent of surviving females. Again, an increased incidence of subcutaneous tumors was observed.

In a study designed to test the carcinogenic effects of 2,4-, 2,6-, and tDNT, 47 percent of male Fischer 344 rats fed 35 mg/kg/day tDNT (about 6.6 mg/kg/day 2,6-DNT) for 1 yr developed hepatocarcinomas, compared with 85 and 100 percent for rats fed 7 and 14 mg/kg/day pure 2,6-DNT, respectively, and none for rats fed 27 mg/kg/day pure 2,4-DNT. This indicates that most of the carcinogenicity of tDNT can be attributed to the 2,6- isomer rather than the 2,4- isomer, and indicates that pure 2,4-DNT is not carcinogenic. However, limitations of the study preclude a definitive statement regarding the carcinogenicity of 2,4-DNT, and the weight-of-evidence for carcinogenicity is best considered to be in Group D.

Increased pectin in the diet of Fischer 344 rats has been shown to cause an increased incidence of hepatic carcinomas following ingestion

of 3.5 mg/kg/day 2,6-DNT for 1 yr. In contrast, rats fed diets low in pectin did not develop any tumors or neoplastic nodules after feeding with 3.5 mg/kg/day 2,6-DNT for 1 yr.

Both 2,6-DNT and tDNT were found to be weak tumor initiators using an in vivo hepatic initiation-promotion assay. The 2,4- isomer was not found to be an initiator, although it was found to be a weak tumor promoter. On the other hand, 2,6-DNT was found to have strong tumor promoting activity.

5. CRITERION FORMULATION

5.1 EXISTING GUIDELINES AND STANDARDS

No standard for exposure to 2,4-DNT in drinking water exists in the United States. However, USEPA (1980a) reports that a Russian study recommends that a maximum permissible concentration in surface waters should be set at 0.5 mg/L for each of the DNT isomers.

The threshold limit value (TLV) for 2,4-DNT recommended by the ACGIH (1986) is 1.5 mg/m^3, with the notation "skin" indicating the possibility of cutaneous absorption. This value represents the highest value to which a worker may be exposed without adverse effects, and it includes dermal exposure for a normal 8-hr workday of a 40-hr week. However, the National Safety Council (NSC 1976) points out that this TLV is valid only for inhalation exposures and suggests that, since cutaneous absorption is the most serious mode of systemic absorption, the TLV is meaningless in the event of skin contact. A TLV-STEL (short-term exposure level) of 5 mg/m^3 has also been set by ACGIH (1986).

Sittig (1981) reports a level of 200 mg/m^3 2,4-DNT as the maximum concentration established by NIOSH from which one could escape within 30 min without suffering any irreversible health effects.

5.2 OCCUPATIONAL EXPOSURE

There are no contemporary studies documenting occupational exposures to 2,4- or 2,6-DNT. However, routine air sampling at an explosives factory recorded atmospheric levels of DNT (isomers not given) in the plant of 0.03 to 0.1 mg/m^3. Air samples taken close to particularly dusty areas ranged from 0.02 to 2.68 mg/m^3 DNT (Woolen et al. 1985). A National Institute for Occupational Safety and Health (NIOSH) health evaluation study reported DNT concentrations (isomers not identified) in workroom air ranging from 0.013 to 0.42 mg/m^3 during an initial survey, and from nondetectable to 0.10 mg/m^3 during a follow-up survey. All DNT air concentrations were below the OSHA standard of 1.5 mg/m^3 (Ahrenholz and Channing 1980). Subchronic and chronic exposures of workers in munitions plants have been reported from the early and mid 1900s (Perkins 1919, McGee et al. 1942). Symptoms recorded following exposure were cyanosis, dizziness, and a tendency to sleep, with headache, dyspnea, and brown urine. Alcoholic subjects were quite sensitive to oxcit. Aching in joints, particularly the knees, was reported, continuing in some cases for several months after exposure. Recovery occurred [illegible] days after exposure, and more quickly if the subject left the area immediately. Other symptoms included vomiting, numbness and tingling, loss of weight, and diarrhea. More advanced symptoms include jaundice and secondary anemia (NSC 1976). No fatal accidents have been recorded. Improvements in hygiene standards have nearly eliminated the acute effects of DNT exposures in modern industry.

A study of cohorts of workers exposed to DNT in two munitions plants operating in the 1940s and 1950s indicated an increase in ischemic heart disease over that seen in white males in the United States and in persons living in the vicinity of the two plants (Levine et al 1986). At both plants mortality from ischemic heart disease was less than expected during the first 15 yr following exposure, increasing only in later years. The data suggest a correlation between mortality and length and intensity of exposure to DNT.

5.3 PREVIOUSLY CALCULATED CRITERIA

Based on their acute and chronic aquatic toxicity studies, and using the 1980 USEPA methodology for estimating aquatic water quality criteria, Bailey et al. (1984) estimate a maximum allowable concentration for 2,4-DNT of 8.1 mg/L and an allowable 24-hr average concentration of 0.12 mg/L.

In the ambient water quality criteria document for dinitrotoluene (USEPA 1980a) a criterion has not been estimated that would protect aquatic life and its uses in either fresh- or saltwater. However, in their draft criteria document (USEPA 1979), 620 μg/L as a 24-hr average and 1000 μg/L as a maximum concentration are proposed.

Ellis et al. (1980) estimated a human health criterion for 2,4-DNT based on their studies demonstrating that it produces mammary tumors in female rats. A value of 1.152 μg/L in drinking water was suggested to maintain a 10^{-5} level of protection. In making this estimate, they used the one-hit model of the USEPA (1979) and assumed that the length of exposure was 728 days, the lifetime of the rat was 770 days, and the female weight was 0.285 kg. They also estimated a bioconcentration factor (BCF) of 18.8 L/kg.

Using the same studies, the USEPA (1980a) estimated an identical water quality criterion of 1.1 μg/L to maintain a 10^{-5} level of risk. However, they used the linearized multistage model (USEPA 1980b) and based their estimate on the combined incidence of mammary tumors, hepatocellular carcinomas, and hepatic neoplastic nodules in female Charles River rats. In addition, they assumed the length of exposure was 720 days, the lifetime of the rat was 750 days, the weight was 0.464 kg, and the BCF was 3.8 L/kg.

The USABRDL recommended a value of 0.74 μg/L as an interim standard for protection of human health (Dacre 1980; U. S. Army 1982, 1983). This value was taken directly from the draft water quality criteria document for 2,4-DNT (USEPA 1979). This value was subsequently revised by the USEPA to the value of 1.1 μg/L as reported in the final criteria document (USEPA 1980a).

5.4 AQUATIC CRITERIA

A brief description of the methodology proposed by the U.S. Environmental Protection Agency (USEPA) for the estimation of water quality criteria suitable for the protection of aquatic life and its uses is presented in Appendix A. The aquatic criteria, as proposed by USEPA, consist of two values, a Criterion Maximum Concentration (CMC) and a Criterion Continuous Concentration (CCC) (Stephan et al. 1985). The CMC is equal to one-half the Final Acute Value, while the CCC is equal to the lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value.

Data available for calculating a Final Acute Value for 2,4-DNT do not meet all the requirements specified by the USEPA guidelines (Stephan et al. 1985); i.e., only seven of the appropriate families of aquatic test animals have been used in acute LC_{50} tests rather than the eight families required by the guidelines (see Appendix A). However, since the data generated by these toxicity tests are uniform in their assessment of the degree of toxicity of 2,4-DNT, a freshwater Final Acute Value was estimated using the formulae provided in the USEPA guidelines (Stephan et al. 1985).

The steps used for calculating the Final Acute Value are shown in Table 20. Since nine families of aquatic test animals were used in the acute toxicity testing, even though they do not represent the necessary eight families required by the guidelines, the value of N is taken as equal to 9. The results of flow-through tests with measured concentrations are available for six of the families tested, and these values have been used for calculation of a Final Acute Value, as well as the results from static tests for the other three families. A Final Acute Value of 11.02 mg/L is estimated. It should be emphasized that this value represents an interim value because, as noted above, in accordance with the USEPA guidelines (Stephan et al. 1985), a final freshwater animal acute value for 2,4-DNT cannot be calculated until one additional family in any order of insect or any phylum not already represented is tested for 2,4-DNT toxicity. Insufficient data are available to calculate a Final Acute Value for 2,6-DNT.

According to USEPA guidelines for water quality criteria, acute and chronic flow-through tests using measured concentrations for three species of organisms are required to estimate an acute/chronic ratio. The results of the chronic toxicity tests of Bailey et al. (1984) are given in Table 21, as well as the results of acute flow-through tests using measured concentrations, performed in the same laboratory (Liu et al. 1983). The geometric mean of the acute/chronic ratios is 54.3, resulting in a Final Chronic Value of 0.20 mg/L. One study indicates that toxicity of 2,4-DNT to bluegill sunfish is affected by water quality parameters, temperature in particular. However, the USEPA guidelines require evidence that the toxicity of a chemical to two or more species is similarly related to a water quality characteristic in order to justify estimating a water quality criterion that is water-quality-dependent.

TABLE 20. CALCULATIONS FOR FINAL ACUTE VALUE (FAV) OF 2,4-DNT[a,b]

Rank (R)	GMAV[c]	ln GMAV	(ln GMAV)^2	P = R/(N+1)[d]	$\sqrt{P}$
4	25	3.2189	10.3613	0.4	0.6325
3	22.5	3.1135	9.6939	0.3	0.5477
2	16.0	2.7726	7.6873	0.2	0.4472
1	13.9	2.6319	6.9269	0.1	0.3162
Sum:		11.7369	34.6694	1.0	1.9436

a. 2,4-DNT = 2,4-dinitrotoluene.
b. Based on calculation methods discussed in Stephan et al. 1985.
c. GMAV = genus mean acute value in mg/L.
d. P = probability for each GMAV; R = rank of four lowest GMAVs; N = 9.

$$S^2 = \frac{\Sigma[(\ln GMAV)^2] - (\Sigma(\ln GMAV)^2/4)}{\Sigma(P) - (\Sigma\sqrt{P})^2/4}$$

$$L = [\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P}))]/4 ,$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A .$$

$$S^2 = \frac{34.6694 - (11.7369)^2/4}{1.0 - (1\ 9436)^2/4} = 4.1493;\ S = 2.0370 .$$

$$L = (11.7369 - (2.0370)(1.9436))/4 = 1.9444 ,$$

$$A = (2.0370)(\sqrt{0.05}) + (1.9444) = 2.3999 ,$$

$$FAV = e^{2.3999} = 11.0206 \text{ mg/L}.$$

TABLE 21. SUMMARY OF 2,4-DNT[a] CHRONIC AQUATIC TOXICITY STUDIES[b]

Species	Method	Endpoint	Limits (mg/L)	Geometric Mean (mg/L)	Acute Value (mg/L)	Acute/ Chronic Ratio
Salmo gairdneri	ELS[d]	Fry growth	0.56 - 1.17	0.81	13.9	17.16
Pimephales promelas	CS[e]	Reproduction	0.28 - 0.62	0.42	36.1	85.95
Daphnia magna	CS[e]	Reproduction	0.19 - 0.40[f]	0.28	30.4	108.57

a. 2,4-DNT = 2,4 dinitrotoluene.
b. From Bailey et al. 1984.
c. From Liu et al. 1983.
d. ELS = early-life-stage study
e. CS = chronic study
f. The validity of this value is questionable due to the low reproduction reported in control populations.

USEPA guidelines (Stephan et al. 1985) state that a Final Plant Value is the lowest result from a test with an important aquatic species in which the concentration of test material was measured and the end-point was biologically important. In both the test with the alga Microcystis aeruginosa (Liu et al. 1983) and with the flowering plant Lemna perpusilla Torr. (Schott and Worthley 1974), growth was significantly depressed at 0.5 mg/L 2,4-DNT, and no significant effects were seen at 0.1 mg/L. Following the USEPA guidelines, a Final Plant Value would be 0.5 mg/L 2,4-DNT. No comparable data are available for 2,6-DNT, and thus no Final Plant Value can be presented for this isomer.

Although no steady-state or 28-day bioaccumulation studies are available for the DNT isomers, short-term bioconcentration studies with 2,4-DNT indicate that it is only slightly accumulated in edible tissues, with bioconcentration factors (BCF) of 4 and 11 reported for bluegill muscle (Liu et al. 1983; Hartley 1981, respectively). Rapid clearance (1 to 4 days) of 2,4-DNT occurs when bluegills are placed in a 2,4-DNT-free environment. An estimated BCF for all six isomers of DNT, based on the octanol/water partition coefficient, is 31.83 (Liu et al. 1983). Ellis et al. (1979) report a calculated value of 18.8 for 2,4-DNT. No maximum permissible tissue concentration is available for 2,4-DNT or 2,6-DNT, nor are there any chronic wildlife feeding studies from which to estimate acceptable daily intake. Therefore, no Final Residue Value can be calculated for either isomer.

In summary, the water quality criteria to protect freshwater aquatic organisms and their uses, as proposed by USEPA, consist of two values, a Criterion Maximum Concentration (CMC) and a Criterion Continuous Concentration (CCC) (Stephan et al. 1985). The CMC is equal to one-half the Final Acute Value, while the CCC is equal to the lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value. Although insufficient data are available to calculate a criterion as described in the USEPA guidelines, an interim CMC of 5.5 mg/L 2,4-DNT and an interim CCC of 0.20 mg/L 2,4-DNT are suggested. At present, no CMC or CCC can be calculated for 2,6-DNT; however, based on only a few studies, it appears that the 2,6- isomer is approximately twice as toxic as the 2,4- isomer to aquatic organisms (see Tables 4 and 5).

There are no data available documenting the acute or chronic toxicity of 2,4- or 2,6-DNT to saltwater organisms.

5.5 HUMAN HEALTH CRITERIA

Two studies are available documenting the oncogenic effects of 2,4-DNT in rats and mice. The NCI (1978) report indicates that 2,4-DNT produces benign subcutaneous tumors in male rats and mammary fibroadenomas in female rats when present as 0.02 or 0.008 percent of the diet for 18 months. No evidence of treatment-related carcinomas was seen in rats, and no evidence of any treatment-related tumors, benign or malignant, was seen in mice of either sex.

In the Ellis et al. (1979) study, a significant increase over controls in the incidence of hepatocellular carcinomas and hepatic neoplastic nodules was observed in female rats following administration of 45.3 mg/kg/day 2,4-DNT in the diet for 2 yr. Male and female rats also experienced a statistically significant increase in benign subcutaneous and mammary tumors. Ellis et al. (1979) also reported an increased incidence of renal tumors in male Charles River CD-1 mice at the intermediate dose tested (95 mg/day 2,4-DNT) after 2 yr of feeding. There was only a slight increase in the incidence of renal tumors at the highest dose tested (900 mg/kg/day), but this was probably a result of the high mortality rate at this level of intake.

In the study of Leonard et al. (1987), male Fischer 344 rats were fed 27 mg/kg/day pure 2,4-DNT for 1 yr. There was no evidence of carcinogenicity; however, limitations of the study preclude a definitive statement regarding the carcinogenicity of 2,4-DNT, and the weight of evidence for carcinogenicity is best considered to be in the USEPA Group D category. In addition, there are no studies available documenting the systemic toxicity of pure 2,4-DNT, and thus no acceptable daily intake can be calculated.

The water quality criterion for 2,4-DNT is derived from the study by Ellis et al. (1979), in which an increased incidence of hepatocellular carcinomas and hepatic neoplastic nodules was observed in female Charles River CD rats. These data resulted in the highest potency factor when the carcinogenic and related tumor data were compared between male and female rats and mice. It should be noted that the 2,4-DNT used in the Ellis et al. (1979) bioassay was 98 percent pure with the remaining 2 percent comprised of predominantly 2,6-DNT. The effect of the 2,6-DNT isomers on the carcinogenic activity of 2,4-DNT was not directly assessed, but by analogy to the studies on the effects of 2,6-DNT on the results of the

The methodology outlined by the USEPA for the development of ambient water quality criterion for the protection of human health is briefly summarized in Appendix B. There are several mathematical methods available to determine a threshold for carcinogens (USEPA 1980b). Therefore, the recommended criterion for the protection of human health is zero. However, zero concentration in some cases may not be feasible. Therefore, USEPA presented a range of concentrations associated with upper-bound risks of 10^{-5} to 10^{-7}. For example, the risk of an additional case of cancer may occur in a population of 100,000. The USEPA (1980b) has adopted the model [redacted] USEPA 1980b (GLOBAL79), to estimate the water concentrations that cause a lifetime carcinogenic risk of [redacted] multistaged model. The water concentrations of 2,4-DNT that are estimated to cause a lifetime carcinogenic risk of 10^{-5} to 10^{-7} have been calculated with the GLOBAL79 model using the Howe program of Howe and Crump (1982). A summary of the pertinent data used in calculating human health risk for 2,4-DNT is presented in Table 22. The oncogenicity data utilized for the calculations are listed in Table 22.

$$q_1^*(A) = \frac{95\% \text{ upper confidence interval}}{\text{MLE}}$$

$$q_1^*(H) = q_1^*(A) \times \left(\frac{W_H}{W_A}\right)^{0.33} \times \frac{L}{L_e}$$

$$q_A = q_1^* H \cdot \quad \cdot \quad B \cdot F$$

$$= \quad \cdot \quad \cdot \quad B \cdot F$$

where

AD-A188 713 WATER QUALITY CRITERIA FOR 24-DINITROTOLUENE AND 26-DINITROTOLUENE(U) OAK RIDGE NATIONAL LAB TN BIOLOGY DIV E L ETNIER AUG 87 ORNL-6312 2/2

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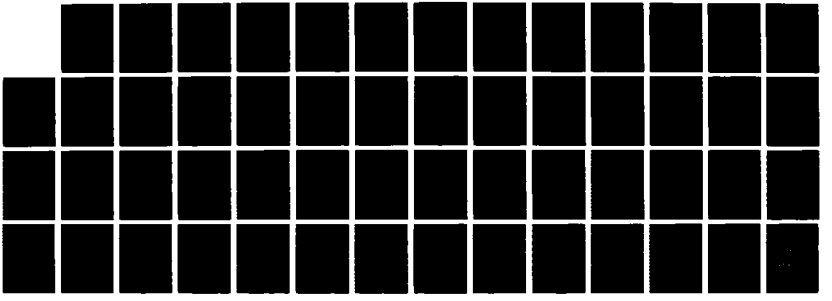

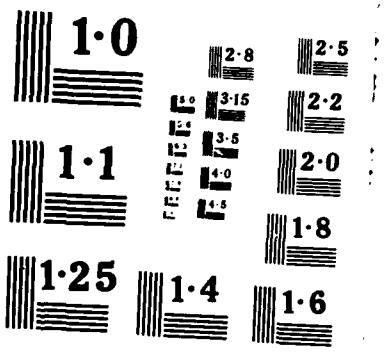
1·0
1·1
1·25
1·4
1·6
1·8
2·0
2·2
2·5
2·8
3·15
3·5
4·0
4·5

TABLE 22. SUMMARY OF PERTINENT DATA FOR CALCULATING THE LIFETIME CARCINOGENIC RISK OF 2,4-DINITROTOLUENE[a]

Dose (mg/kg/day)	Incidence (No. responding/No. tested)
0.0	0/19
0.71	2/33
5.1	3/23
45.3	23/35

a. The water quality criterion for 2,4-dinitrotoluene is based on the induction of hepatocellular carcinomas and hepatic neoplastic nodules in female Charles River CD rats (Ellis et al. 1979).

Leonard et al. (1987) reported hepatic neoplastic nodules in 5, 53, 90, and 79 percent of male Fischer 344 rats following 1-yr feeding of 27 mg/kg/day pure 2,4-DNT, 35 mg/kg/day tDNT, 7 mg/kg/day 2,6-DNT, and 14 mg/kg/day 2,6-DNT, respectively. Animals with only hepatocarcinomas represented 0, 47, 85, and 100 percent, respectively, of the test groups. Multiple carcinomas were frequently found in tDNT- and 2,6-DNT-fed rats, and 10 percent of the animals fed tDNT or 7 mg/kg/day 2,6-DNT had cholangiocarcinomas. Control rats showed no incidence of hepatic neoplastic nodules or hepatic carcinomas.

Since only two concentrations of 2,6-DNT were tested in the study by Leonard et al. (1986), and the incidence of hepatic neoplastic nodules or hepatic carcinomas was 100 percent in rats treated at both doses, there is no apparent dose response if both lesions are considered. Therefore, the water quality criterion for 2,6-DNT is derived from the data of Leonard et al. (1987) showing an increased incidence of hepatic carcinomas in male Fischer 344 rats. It should be noted that the studies by Goldsworthy et al. (1986) indicate that diet can affect the carcinogenicity of the DNT isomers, enhancing the metabolism and hepatic covalent binding of 2,6-DNT in particular. Components in the diet used in the Leonard et al. (1986) study may have influenced the carcinogenicity of the isomer. However, 2,6-DNT is unquestionably a potent hepatocarcinogen, and a criteria based on this study will give a conservative estimate of the range of acceptable cancer risk. It should be noted that the studies by Goldsworthy et al. (1986) indicate that diet can affect the carcinogenicity of the DNT isomers, enhancing the metabolism and hepatic covalent binding of 2,6-DNT in particular. Components in the diet used in the Leonard et al. (1986) study may have influenced the carcinogenicity of the isomer. However, 2,6-DNT is unquestionably a potent hepatocarcinogen and criteria based on this study will give a

conservative estimate of the range of acceptable cancer risk. Table 23 lists the oncogenicity data used for the calculation. The formulae outlined above were used,

BCF = Bioconcentration factor = 18.8 L/kg (Ellis et al. 1979)

2 = human water consumption, L/day

0.0065 = human fish consumption, kg/day

95% Upper confidence interval = 6.73184×10^{-3}

MLE = Maximum likelihood estimate = 1.60269×10^{-2} mg/kg/day

$q_1^*(A)$ = Carcinogenic potency factor for animals = 4.20×10^{-1} $(mg/kg/day)^{-1}$

$q_1^*(H)$ = Carcinogenic potency factor for humans = 4.827 $(mg/kg/day)^{-1}$

W_H = Average weight of humans = 70 kg

W_A = Average weight of experimental animals = 0.350 kg (USEPA 1986)

l_e = Duration of exposure = 365 days

L = Lifespan of test animal = 730 days

C_A = Concentration of 2,6-dinitrotoluene in water, calculated to keep the lifetime cancer risk below 10^{-5} = 0.068 $\mu g/L$

C_O = Concentration in organisms only, calculated to keep the lifetime cancer risk below 10^{-5} = 1.19 $\mu g/L$

TABLE 23. SUMMARY OF PERTINENT DATA FOR CALCULATING THE LIFETIME CARCINOGENIC RISK OF 2,6-DINITROTOLUENE[a]

Dose (mg/kg/day)	Incidence (No. responding/No. tested)
0.0	0/20
7	17/20
14	19/19

a. The water quality criterion for 2,6-dinitrotoluene is based on the induction of heptotocellular carcinomas in male Fischer 344 rats (Leonard et al. 1987).

The recommended criteria to achieve a risk of 10^{-5}, 10^{-6}, or 10^{-7} for 2,6-dinitrotoluene are 68.3, 6.8, and 0.68 ng/L, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 1.?, 0.12, and 0.012 $\mu g/L$, respectively.

5.6 RESEARCH RECOMMENDATIONS

The following research recommendations are intended to fill the data gaps necessary to meet the USEPA requirements for generating aquatic water quality criteria for 2,4-dinitrotoluene and 2,6-dinitrotoluene.

1. An acute aquatic toxicity test for 2,4-DNT using flow-through conditions, with measured concentrations, for any order of insect, e.g., the mayfly nymph Hexagenia sp., or any other phylum not previously tested.

2. Acute toxicity tests for 2,6-DNT using flow-through conditions with measured concentrations for families other than Daphnidae and Cyprinidae in order to satisfy the minimum database requirements of the USEPA (Stephan et al. 1985).

3. Chronic flow-through tests for 2,4-DNT with Daphnia magna in which reproduction and survival of control organisms meet current test standards.

4. Chronic flow-through tests for 2,6-DNT using measured concentrations for at least three species of aquatic animals, provided that one is an invertebrate species and one a sensitive freshwater species. These tests could include life-stage tests with Pimephales promelas and Salmo gairdneri, and a 21-day life-cycle test with Daphnia magna.

5. Steady-state or 28-day bioaccumulation studies for both 2,4- and 2,6-DNT.

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7. GLOSSARY

ALT	Aerated lagoon treatment
AAP	Army Ammunition Plant
ADI	Acceptable daily intake
2A4AABA	2-Amino-4-acetylaminobenzoic acid
4Ac2NT	4-Acetamido-2-nitrotoluene
2A4NBA	2-Amino-4-nitrobenzoic acid
2A4NBAlc	2-Amino-4-nitrobenzyl alcohol
4A2NBAlc	4-Amino-2-nitrobenzyl alcohol
2A4NT	2-Amino-4-nitrotoluene
4A2NT	4-Amino-2-nitrotoluene
4Ac2NBA	4-(N-acetyl)amino-2-nitrobenzoic acid
BCF	Bioconcentration factor
BOD	Biological oxygen demand
COD	Chemical oxygen demand
4AcA2NBA	4-Acetylamino-2-nitrobenzoic acid
4AcA2NT	4-Acetylamino-2-nitrotoluene
2,4ANBAlc	2,4-Aminonitrobenzyl alcohol
2,4-DABAlc	2,4-Diaminobenzyl alcohol
2,4DAT	2,4-Diaminotoluene
DCNP	Dichloronitrophenol
DMSO	Dimethyl sulfoxide
4,4'Az	2,2'-Dinitro-4,4'-azoxytoluene
2,2'Az	4,4'-Dinitro-2,2'-azoxytoluene
2,4DNBA	2,4-Dinitrobenzoic acid
2,4DNBAlc	2,4-Dinitrobenzyl alcohol

2,4DNBAl	2,4-Dinitrobenzyl aldehyde
2,4DNBAlcG	2,4-Dinitobenzyl alcohol glucuronide
DNT	Dinitrotoluene
dpm	Disintegrations per minute
ECD	Electron-capture detection
EC_{50}	Effective concentration causing 50 percent death (based on immobilization)
FEL	Frank effect level
GC	Gas chromatography
HPLC	High pressure liquid chromatography
2HA4NT	2-Hydroxylamino-4-nitrotoluene
4HA2NT	4-Hydroxylamino-2-nitrotoluene
i.p.	Intraperitoneal
LC_{50}	Lethal concentration causing 50 percent death
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
MS	Mass spectroscopy
NIOSH	National Institute for Occupational Safety and Health
2N4NT	2-Nitroso-4-nitrotoluene
4N2NT	4-Nitroso-2-nitrotoluene
NOAEL	No observed adverse effect level
NOEC	No observed effects concentration
NOEL	No observed effect level
OECD	Office of Economic and Community Development
PACT	Powdered Activated Charcoal Treatment
ppb	Parts per billion
ppm	Parts per million

ppt	Parts per trillion
PCP	Pentachlorophenol
PID	Photoionization detection
PACT	Powdered activated carbon treatment
TDA	Toluene diamine
tDNT	Technical grade dinitrotoluene
TEA	Thermal energy analyzer
TT	Toxicity threshold
TNT	Trinitrotoluene
UDS	Unscheduled DNA synthesis
UV	Ultraviolet
USAMBRDL	U.S. Army Medical Bioengineering Research and Development Laboratory

APPENDIX A

SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect aquatic life with emphasis on the specific regulatory needs of the U.S. Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C. E. Stephan, D. I. Mount, D. J. Hansen, J. H. Gentile, G. A. Chapman, and W. A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species at all times was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species is very sensitive. The small fraction is set at 0.05, because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from

acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors, such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived; however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative; much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.

3. Definition of the material should include an operational analytical component. It is also necessary to reference or describe analytical methods that the term is intended to denote. Primary

requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

NOTE: Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants; (b) FDA action levels (FDA Guidelines Manual); and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms

2. All data used should be available in typed, dated, and signed hardcopy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.

3. Questionable data, whether published or not, should not be used.

4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.

5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentration of test material in test solutions were measured using acceptable analytical methods.

6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:

a. the family Salmonidae in the class Osteichthyes;

b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish);

c. a third family in the phylum Chordata (e.g, fish or amphibian);

d. a planktonic crustacean (e.g, cladoceran or copepod);

e. a benthic crustacean (e.g, ostracod, isopod, or amphipod);

f. an insect (e.g., mayfly, midge, stonefly);

g. a family in a phylum other than Arthropoda or Chordata (e.g, Annelida or Mollusca); and

h. a family in any order of insect or any phylum not represented.

2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.

3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.

4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly, if all required data are not available a numerical criterion should not be derived except in special cases. For example, even if sufficient acute and chronic data are available, it may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.

2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).

3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.

4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L), should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.

5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:

   a. Tests with daphnids and other cladocerans should be started with organisms <24 hr old, and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} is not available from a test, the 48-hr LC_{50} should be used in place of the desired 48-hr EC_{50}. An EC_{50} or LC_{50} of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.

   b. The result of tests with all other aquatic animal species should be the 96-hr EC_{50} value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} value is not available from a test, the 96-hr LC_{50} should be used in place of the desired EC_{50}.

   c. Tests with single-cell organisms are not considered acute tests, even if the duration was $\leq$96 hr.

   d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.

6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.

7. If the data indicate that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV, because a species can only be considered protected from acute toxicity if all life stages are protected.

8. Consider the agreement of the data within and between species. Questionable results in comparison with other acute and chronic data for the species and other species in the same genus probably should not be used.

9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentrations of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

   NOTE: Data reported by original investigators should not be rounded off, and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.

11. Order the GMAVs from high to low, and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.

12. Calculate the cumulative probability (P) for each GMAV as R/(N+1).

13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).

14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^{A}$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.

16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.

3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.

4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

5. Similarly normalize the values of the water quality characteristic for each species individually.

6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).

9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.

10. For each species calculate the SMAV using: $SMAV = e^{Y}$.

11. Obtain the FAV at Z by using the procedure described in Section 5 (Nos. 10-14).

12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.

13. The Final Acute Equation is written as: $FAV = e^{(V[\ln(\text{water quality characteristic}) + \ln A - V[\ln Z])}$, where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

   NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one; this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.

3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.

5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

   a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young <48 hr old, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young <24 hr old and last for not <21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.

   b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

   c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

      NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be

used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used, because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.

7. If chronic toxicity of the material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.

8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species, and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (Nos. 10-14). Then, go to Section 7 (No. 13).

9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used or acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.

10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.

11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or

decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.

a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be calculated as the geometric mean of all species whose SMAVs are close to the FAV.

b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and saltwater species.

c. If the most appropriate species mean acute-chronic ratios are <2.0, and especially if they are <1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and an FCV probably cannot be calculated.

12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.

13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.

14. Go to Section 9.

8. FINAL CHRONIC EQUATION

1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.

a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.

b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z (see Section 6, No. 13) by the Final Acute-Chronic Ratio.

c. Use V = pooled acute slope (see Section 6, No. 13) as L = pooled chronic slope.

d. Go to Section 8, No. 2, item m.

2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.

a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.

c. Decide whether data for each species are useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.

d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.

e. Similarly normalize the values of the water quality characteristic for each species individually.

f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality

characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.

i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.

j. For each species calculate a SMCV at Z as the antilog of Q ($SMCV = e^{Q}$).

k. Obtain the FCV at Z by using the procedure described in Section 5 (Nos. 10-14).

l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.

m. The Final Chronic Equation is written as: $FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$, where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.

2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.

3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which

concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) an FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11, because a Final Residue Value cannot be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.

3. If a maximum permissible tissue concentration is available for a substance (e.g, parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.

   a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached; (b) highest BCF obtained, if

apparent steady-state was not reached; and (c) projected steady-state BCF, if calculated.

b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.

c. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.

d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.

e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, in which case the BCF for the longest exposure should be used.

4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.

b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.

a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage

of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.

b. Calculate the geometric mean normalized BCF.

c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.

- For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.
- For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).
- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.

3. The Criterion Continuous Concentration (CCC) is equal to the lowest of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final

Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.

4. Round both the CCC and CMC to two significant figures.

5. The criterion is stated as:

   The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) μg/L more than once every three years on the average and if the one-hour average concentration does not exceed (4) μg/L more than once every three years on the average.

   Here,

   (1) = insert freshwater or saltwater,

   (2) = insert name of material,

   (3) = insert the Criterion Continuous Concentration, and

   (4) = insert the Criterion Maximum Concentration.

13. REFERENCES

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APPENDIX B

SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final US Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect human health and is slanted towards the specific regulatory needs of the U.S. Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

a) Carcinogenicity,
b) Toxicity, and
c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the values represent estimates that would prevent adverse health effects, or for suspect and proven carcinogens, estimates of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAH HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes, including ingestion of contaminated water and edible aquatic and nonaquatic organisms as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish.

Generally, exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to be insignificant; however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions, which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).

2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.

3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used, depending upon lipid solubility and availability of bioconcentration data:

1. For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980).

   Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCF_{avg} = BCF_{sp} \times \frac{3.0\%}{PL_{sp}} ,$$

   where BCF_{sp} is the bioconcentration factor for an aquatic species and PL_{sp} is the percent lipids in the edible portions of that species.

2. Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

   for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent

lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

3. For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity; synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects, is selected for criteria formulation.

3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to 10^{-5} (one additional case of cancer in populations ranging from ten

million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1 METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTHRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific lifetime carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065\ BCF)}$$

where

- C = ambient water concentration;
- PR = the probable risk (e.g., 10^{-5}; equivalent to one case in 100,000);
- BCF = the bioconcentration factor; and
- q_1^* = a coefficient, the cancer potency index (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = \frac{q_1^* \times C\ (2 + 0.0065\ BCF)}{70}$$

or

$$PR = \frac{q_1^* \times 2C + (0.0065\ BCF \times C)}{70}$$

where 2C is the daily exposure resulting from drinking 2 liters of water per day and (0.0065 BCF x C) is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* X ,$$

where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X} .$$

showing that the coefficient q_1^* is the ratio of risk to dose, an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5}, which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared with the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then RR(X) = 5). In such cases the "excess" relative cancer risk is expressed as RR(X) - 1, and the actual numeric, or proportional, excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(control) \ .$$

Using the standard risk/dose equation

$$PR(X) = b \times X$$

and substituting for PR(X):

$$[RR(X) - 1] \times PR(control) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(control)}{X} \ ,$$

where b is equal to the carcinogenic potency or q_1^*.

3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.

2. The data set giving the highest index of carcinogenic potency (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.

3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.

4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the 2/3 power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}$$

2. If the dose (s) is given as mg per kg of body weight:

$$S = \frac{m}{W}$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times w)}{W^{2/3}}$$

or

$$X = s \times W^{1/3} .$$

3. The dose must also be normalized to a lifetime average exposure. For a carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is l_e, and the total length of the experiment is L_e, then the lifetime average exposure (X_m) is

$$X_m = \frac{l_e \times m}{L_e \times W^{2/3}} .$$

4. If the duration of the experiment (L_e) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of $(L/L_e)^3$ to adjust for an age-specific increase in the cancer rate.

5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = ppm \times F \times r ,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man; 0.05 for a 0.35-kg rat; and 0.13 for a 0.03-kg mouse).

Substituting (ppm x F) for m and fW for F, the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3} .$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol and is absorbed proportionally to to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption, which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t;d) = 1 - \exp\{-g(d)H(t)\},$$

where P(t;d) is the probable response for dose d and time t, g(d) is the polynomial function defining the effect of dose level, and H(t) the effect of time:

$$g(d) = \sum_{i=0}^{a} \alpha_i d^i$$

$$H(t) = \sum_{i=0}^{b} \beta_i t^i$$

(with α and $\beta \geq 0$, and $\Sigma \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each α as a multiplicative constant (Crump 1980):

$$p(d/t) = 1 - \exp\left(- \sum_{i=0}^{a} \alpha d^i \right) ,$$

or as given in the EPA guidelines (USEPA 1980):

$$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

where P(d) is the lifetime risk (probability) of cancer at dose d.

For a given dose the excess cancer risk A(d) above the background rate P(o) is given by the equation:

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)}$$

where,

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \ldots + q_k d^k)] .$$

Point estimates of the coefficients $q_1 \ldots q_k$ and consequently the extra risk function A(d) at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function A(d) has approximately the form:

$$A(d) = q_1 \times d .$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R. Thus, A(d) and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1. This is accomplished by using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^*, the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_0 - L_1) = 2.70554 ,$$

where L_0 is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$\chi^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i (1 - P_i)}$$

where N_i is the number of animals in the ith dose group, X_i is the number of animals in the ith dose group with a tumor response, P_i is the probability of a response in the ith dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (χ^2) is larger than the cumulative 99 percent point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at and below which the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065 \text{ kg} \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criterion.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

(1) NOEL = No-Observed-Effect-Level,
(2) LOEL = Lowest-Observed-Effect-Level,
(3) LOAEL = Lowest-Observed-Adverse-Effect-Level,
(4) FEL = Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.

2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.

3. If an LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to an NOAEL or an LOAEL.

4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.

5. If for reasonably closely spaced doses only an NOEL and an LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, an NOAEL, an LOAEL, and a clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.

2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.

3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals, with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported, but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criterion (using the uncertainty factor approach). Also, the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to

those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = \frac{TLV \times BR \times DE \times d \times A_A}{(A_O \times SF)}$$

where

BR = daily air intake (assume 10 m^3),
DE = duration of exposure in hours per day,
d = 5 days/7 days,
A_A = efficiency of absorption from air,
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = \frac{CA \times D_E \times d \times A_A \times BR \times 70\ kg}{BW_A \times A_O \times SF)}$$

where

C_A = concentration in air (mg/m^3),
D_E = duration of exposure (hr/day),
d = number of days exposed/number of days observed,
A_A = efficiency of absorption from air,
BR = volume of air breathed (m^3/day),
70 kg = standard human body weight,
BW_A = body weight of experimental animals (kg),
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may, under some circumstances, result in depressed fluid intake which, in

turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant; however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

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MARCH, 1988

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